

Universität Bielefeld

Fakultät für Psychologie und Sportwissenschaft

Abteilung Sportwissenschaft

Arbeitsbereich Sportmedizin

Inagural-Dissertation

zur Erlangung des Doktorgrades

der Naturwissenschaften

(Dr. rer. nat.)

**Effects of Low and High Intensity Exercises on Serum Mean Values of
Myoglobin and Heart-type Fatty Acid-binding Protein in Athletes**

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Bielefeld, im Juli 2010

به نام خداوند جان و خرد

To: My parents

*They, not only gave me life, but also fill it with all the love and affection one
can wish for.*

To: Nahid & Andisheh

A good family is the best basis for a good life.

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1. Introduction

Skeletal muscle is the largest component of adipose tissue free body mass in the human body. [1] This remarkable part of the body consists of many muscles; each is combined by muscle fibers or cells and connective tissues and plays an important role in physiologic processes and energy metabolism. The basic unit of skeletal muscle is the muscle cell or fiber, a long cylindrical structure containing numerous nuclei. Skeletal muscles are voluntary muscles. Without this important part of body, no one can sit, stand, walk or does any activity needed for daily life. Obviously, the skeletal muscles will be damaged often, as they take part in many physical activities. Muscle injuries are one of the most common injuries occurring in sports, with an incidence varying from 10% to 55% of all the sustained injuries. Muscle injury, defined as injury of skeletal muscle involving the rupture of muscle fibres, is one of the most common reasons for consulting a sports medicine specialist. Muscular overuse after high force eccentric muscle action is associated with structural damage of the contractile apparatus which results by releasing muscle proteins. The diagnosis of muscle injuries can be done through several techniques: medical examination, imaging techniques and laboratory tests for blood analysis. Obviously, the best treatment can be given if the injury is recognized in its early stages. The recognition of muscle injury markers is the most effective factor to achieve this goal.

There is a proverb that says: “prevention is better than cure”. It is obviously much better and cost effective to prevent the skeletal muscle injuries than to cure them after their occurrence. As mentioned above, the recognition of muscle injury markers is the most effective factor to diagnose muscle injuries and is also the most cost effective to prevent of worsen of muscle injuries.

Muscle damage after exercise results in a substantial increase in myocellular protein levels in the blood. [2] Exercise induced muscle damage is mainly caused by strong muscle contraction during intense exercise. In recent decades, some

muscle injury markers have been assessed to indicate the skeletal muscle injuries. These markers, rather than other techniques, most probably, also provide trainers with information about muscle damage in sportsmen and women.

Accordingly, due to those above mentioned advantages, the biochemical and physiological characteristics of some of muscle injury markers have been reviewed and summarized in the present study. Subsequently two of those markers were evaluated after the low and high intensity exercises in well trained athletes.

2. Literature

There are about 650 muscles in the human body. [3] Muscle tissue is classified into three types:

Skeletal muscles: Skeletal muscle tissue is named for its location, attached to bones. All skeletal muscle fibers are not alike in structure or function. Skeletal muscles vary considerably in size, shape and arrangement of fibers. An individual skeletal muscle may be made up of hundreds, or even thousands of muscle fibers bundled together and wrapped in a connective tissue covering.

Cardiac muscle: Cardiac muscle tissue forms the bulk of the wall of the heart. Like skeletal muscle tissue, it is striated.

Smooth muscles: Smooth muscle tissue is located in the walls of hollow internal structures such as blood vessels, the stomach, intestines, and urinary bladders.

All three types possess the basic characteristics of muscle tissue:

Extensibility: The ability to stretch

Elasticity: The ability to return to normal length when the stretching force is removed

Excitability: The ability to be excited by stimuli

Contractility: The ability to apply tension when stimulated. [4]

Each muscle is especially suited in both structure and function to its particular task.

Skeletal muscle is a remarkable tissue. Muscle cells are specialized to contract. Because of its ability to shorten and produce force, we are able to breathe, walk and perform all the tasks and activities required in our daily lives. [5] For a human, as for other animals, to move is to survive. And, of course, no muscle contractions

are more important than those responsible for the simple movements of breathing. [6] Skeletal muscles attach at both ends either directly or indirectly to the skeletal system. When the muscle contracts it does not pull mostly on the distal end, but rather it pulls toward its middle, exerting equal force on the attachments at both ends. Which bone moves as a result of the force depends on the relative stability of the bones at the time that is determined, to a large degree, by muscles acting as stabilizers. The muscles vary in their size and shapes and arrangement of fibers. The basic unit of skeletal muscle is the muscle cell or fiber, a long cylindrical structure containing numerous nuclei. An individual skeletal muscle may be made up of hundreds, or even thousands, of muscle fibers bundled together and wrapped in a connective tissue covering. Each muscle is surrounded by a connective tissue sheath called the epimysium. Fascia, connective tissue outside the epimysium, surrounds and separates the muscles. Perimysium is connective tissue that wraps bundles of muscle fibers, the boundless being known as fascicles. Endomysium is connective tissue that wraps each individual muscle fiber. The endomysium and perimysium are relatively thin and consist mainly of reticular fibers. The epimysium is thicker than the intramuscular sheaths and consists of both collagen and reticular fibers. [7] The most obvious structures within the muscle fiber are the myofibrils, which are the units responsible for contraction and relaxation of the fiber. Each myofibril is from 1 to 2 μm in diameter and as long as the entire cell and is separated from its neighbors by mitochondria and the sarcoplasmic and transverse tubular systems; in a fiber of 50 μm diameter, there are up to 8000 myofibrils. Most of the interior of muscle fiber is made up of myofibrils, the protein contractile elements of the cell. About 90% of the substances of the myofibrils, consist of boundless of myofilaments, is represented by the three proteins: myosin, actin, and tropomyosin. When viewed longitudinally under the microscope, each fiber appears as a series of alternating light and dark striations.

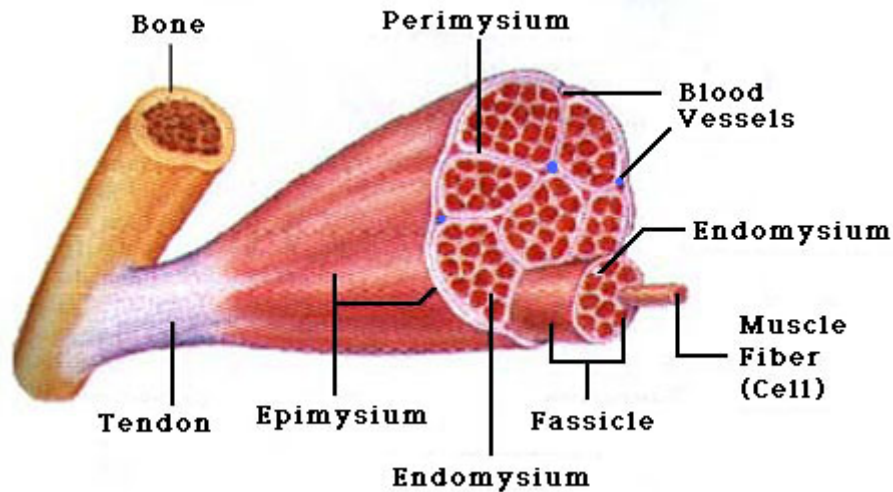


Figure 1: Structure of skeletal muscle (Reproduced from: <http://people.eku.edu/ritchisong/RITCHISO/301notes3.htm>)

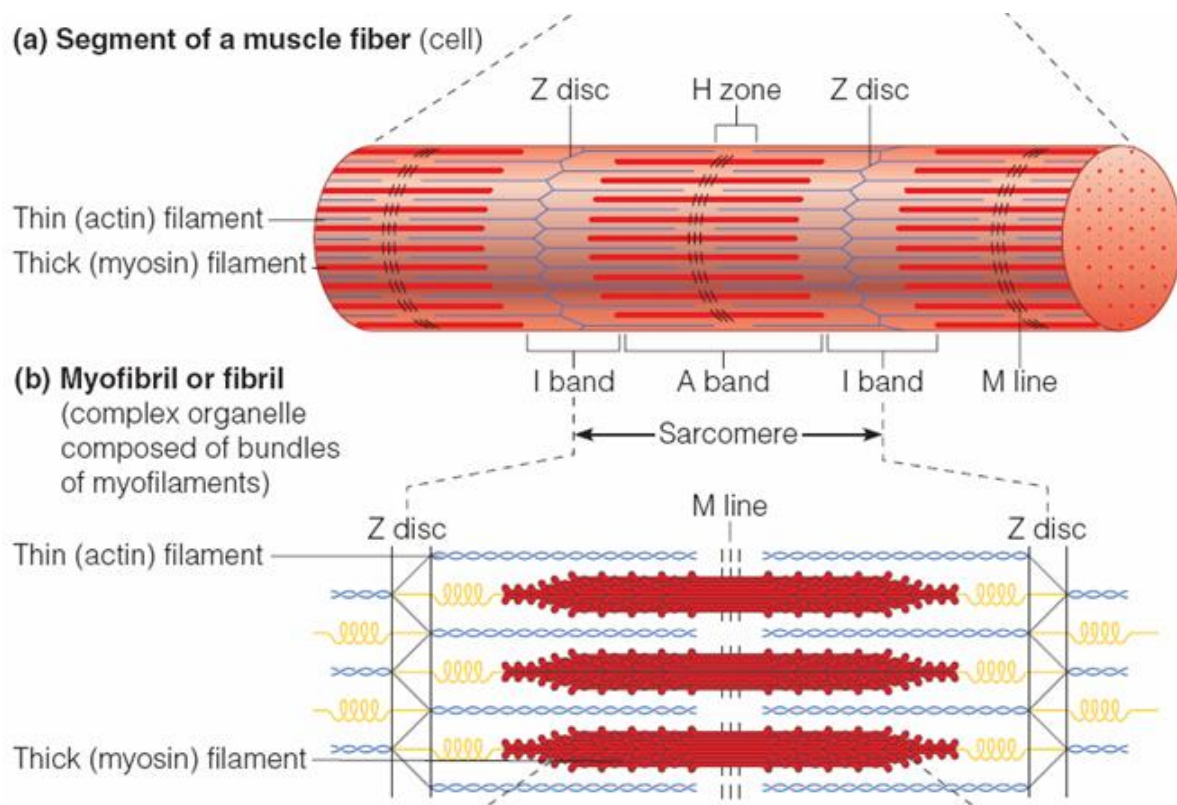


Figure 2: A morphological diagram of striated muscle (Reproduced from:

www.shoppingtrolley.net/skeletal%20muscle.shtml)

A muscle can contract with varying degrees of force at any particular time, and the amount of force is voluntarily controlled. The force of contraction at a given time is controlled by two variables:

- The number of motor units that are activated
- The stimulating frequency or rate

Skeletal muscle fibers are not all the same.

Type I fibers: These fibers, also called slow twitch or slow oxidative fibers, are red and contain large amounts of myoglobin (Mb), many mitochondria and many blood capillaries. They have a slow contraction velocity, very resistant to fatigue and a high capacity to generate Adenosine Triphosphate (ATP) by oxidative metabolic processes. These fibers are suited for endurance trainings. Such fibers are found in large numbers in the postural of the neck.

Type IIa fibers: These fibers, also called fast twitch or fast oxidative fibers, are red and contain very large amounts of Mb, very many mitochondria and very many blood capillaries. These fibers have a very high capacity for generating ATP by oxidative metabolic processes. They have a fast contraction velocity and are resistant to fatigue. Such fibers are infrequently found in humans.

Type IIb fibers: These fibers, also called fast twitch or fast glycolytic fibers, are white and contain a low content of Mb, relatively few mitochondria, relatively few blood capillaries and large amounts glycogen. They have low resistance to fatigue.

2.1. Muscle injuries:

Skeletal muscle cells (fibers), like other body cells, are soft and fragile. (SEER's Training Website. www.web-books.com) Muscle injuries are one of the most common traumas occurring in sports, with an incidence varying from 10% to 55% of all the sustained injuries. [8] A variety of injuries occur to individual while involved in different sports and leisure physical activities. Muscle injuries can be classified into two groups, firstly those resulting from direct trauma and secondary those that occur as a result of indirect injury generated by excessive pull on the muscle. [9] Direct trauma to muscle can result from either penetrating or blunt injury. Blunt injury is very common in competitive contact sports such as rugby and

football with the muscle being exposed to direct compressive forces. According to clinical findings, muscle injuries classify into three categories: [10]

- Grade I lesions: Delayed onset muscle soreness and elongation, very small muscle tear.
- Grade II lesions: Fibrillar disruption, evident muscle tear.
- Grade III lesions: Fiber disruption, evident muscle tear.

Muscle injury can be caused by contusion, strain, or laceration. [8] Muscle lacerations are the most uncommon of the muscle injuries occurring in sports, as more than 90% of all sports-related injuries, are either contusion or strains. A muscle contusion occurs when a muscle is subject to a sudden, heavy compressive force, such as a direct blow of the muscle. This kind of muscle trauma typically takes place in contact sports, whereas sprinting and jumping are the most common activities associated with muscle strains. In strains, an excessive tensile force subjected onto the muscle leads to the overstraining of the myofibers and consequently to a rupture. Indirect trauma is a result of excessive strain on the muscle and often occurs in sports involving sprinting and jumping. Some muscles are more susceptible to strain with rectus femoris, semitendinosus, and gastrocnemius being the most frequently strained muscles. One of the key factors in their susceptibility is that they cross two joints resulting in them being subjected to stretch in more than one area. Eccentric exercise is commonly associated with this type of injury. Muscle contusion is second only to strain as the leading cause of morbidity from sport-related injuries. [11] Severity depends on the site of impact, the activation status of the muscles involved, the age of the patient, and the presence of fatigue.

The detection and diagnosis of muscle injuries is usually performed by several techniques: [12]

- Medical examination: The diagnosis of some injuries is done by medical examination. The doctor asks about the symptoms and medical history. [13] Also doctor does a physical exam. In strain injury the injured area will be examined for:
 - Tenderness over the area of the muscle
 - Pain, especially when contracting the muscle
- Imaging techniques: The main objective in imaging an injured muscle is to determine the location and extent of involvement. Because of its multiplanar

capability and high sensitivity to the hemorrhage and edema that result from soft-tissue injury, magnetic resonance (MR) imaging is the technique of choice for imaging muscles, tendons, and ligaments. [14] MR imaging facilitates the diagnostic process primarily by detecting alterations in muscle size or signal intensity. [15] MRI and computed axial tomography (CT) are now applied as the criterion methods for measuring total-body skeletal muscle mass. [16] Within the past decades, it has been established that T2 relaxation time of muscle in proton MR images increases during exercise, returning to resting values within 1 hour postexercise. [17] Sonography is as useful as MRI in depicting acute hamstring injuries and because of lower costs, may be the preferred imaging technique. [18]

- Laboratory tests for blood analysis: Muscular overuse after high force eccentric muscle action is associated with structural damage of the contractile apparatus that can be observed as Z-line steaming and myofibrillar disruption. Mechanical stress is the major contributing factor for inducing muscle injury, which initiates a cascade of processes resulting in skeletal muscle damage and releasing of muscle fiber proteins. [19] Muscle injury markers determined by laboratory tests, are one of the best recognition means of muscle injuries.

Obviously, the best treatment can be given if the injury is recognized in its early stages. The recognition of muscle injury markers is the most effective factor to achieve this goal.

2.2. Muscle injury markers:

Advances in biological markers (biomarkers) have facilitated the assessment of associations between environmental exposures and potential health effects by improving accuracy and precision of measures and helping to identify high-risk groups in exposed populations. Biomarkers can be measures of exposure, measures of susceptibility (showing either increased or decreased susceptibility to disease), or measures of effect, such as the presence of sub clinical disease processes or diseases themselves. [20] A biomarker is a molecule that indicates an alteration of the physiological state of an individual in relation to health or disease state, drug treatment, toxins, and other challenges of the environment. [21] Many diagnostic markers are also biomarkers.

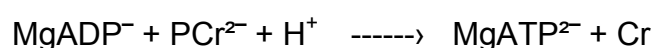
It is well established that periods of increased contractile activity result in significant changes in muscle structure and function. The increased rates of protein degradation and amino acid oxidation that occur during exercise have been attributed to both lysosomal (cathepsin C, D) and non-lysosomal proteases. Lysosomal enzyme activity is generally increased too late in time to account for the loss of myofibrillar protein found immediately post-exercise. Belcastro and colleagues [22] stated that, in vitro, calpain cleaves a variety of substrates: receptor proteins, soluble and membrane associated enzymes, cytoskeletal proteins and myofibrillar proteins. They also proposed that calpain initiates the exercise-induced changes in myofibrillar turnover and organelle disassembly. Although calpain does not degrade actin and myosin, it contributes to their release. [10]

Evaluating of the serum values of muscle injury markers is one of the best indirect indices for assessing the muscle injuries. In recent decades some of blood markers have been used to assess the muscle injuries, each showing the special characteristic issues. The ideal marker should be muscle specific with a high intracellular concentration, and it should be rapidly released in the event of injury and be stable, maintaining its elevated concentration in blood for an adequate time period. It should have a high diagnostic sensitivity, especially in the first few hours after the injury, and have a diagnostic specificity as close to 100% as possible.[23]

In this part of study, some of muscle injury markers will be reviewed and their characteristic issues will be summarized. As creatine kinase (CK) and myoglobin (Mb) are routinely used to evaluate the injury in laboratory tests, [12] these two markers have been reviewed, firstly.

2.2.1. CK:

CK or creatine phosphokinase (CPK) is an enzyme that catalyses the interconversion of phosphocreatine and ATP. During very intense, short-duration activities, CK catalyses the reaction in which the phosphate group from phosphocreatine (PCr) is transferred to adenosine diphosphate (ADP) to synthesis ATP.



CK is a fast ATP-regenerating enzyme. [24] The CK/PCr system is a temporal energy buffer. Total CK levels depend on age, gender, race, muscle mass, physical activity and climatic condition. [25] High CK enzyme activity is detectable in muscle, heart and brain. CK activity is also detectable in serum. The combination of two subunits results in the formation of three types of isoenzymes: CK-MM, CK-BB and the hybrid form of CK-MB are characteristic for muscle, brain and heart, respectively. Figure 1 shows the distribution of CK activities of the human body. [26]

Total CK activities in serum have been investigated extensively to evaluate damage to skeletal and cardiac muscle. CK-MM isoforms into three separate forms can be used as an early detection marker for exercise-induced skeletal muscle injury. [27] D. Kumbhare [28] evaluated the elevation of serum CK concentration as an index of muscle injury using lumbar decompression surgery (LDS) as a model. In lumbar disc surgery (LS), cutting of muscle is avoided. However, injury may be produced in many patients when muscle is stripped from vertebral lamina, retracted away from the spine, and held with surgical clamps. Contributions might also be made by inadvertent muscle tearing, and pressure at retractor contact points. Their findings provided support for the validity of serum CK measurement as an index of skeletal muscle injury caused by LDS.

Although CK is the most common serum marker for skeletal muscle injury, it is not ideal for several reasons, including lack of tissue specificity, inability to reveal damage to specific skeletal fiber types (fast or slow), and inappropriately low values when glutathione concentrations are decreased because of liver or multiple organ failure. [29] Additionally, other causes for low CK activity have been described. In multiple organ failure, tissue factors, such as lysosomal enzymes, that are able to deactivate CK are released into the circulation. The absence of physical exercise in the immobilized critically ill patients contributes to low CK activity in serum. Attention should be paid when interpreting serum CK activity, especially in clinical conditions associated with low extracellular glutathione concentrations.

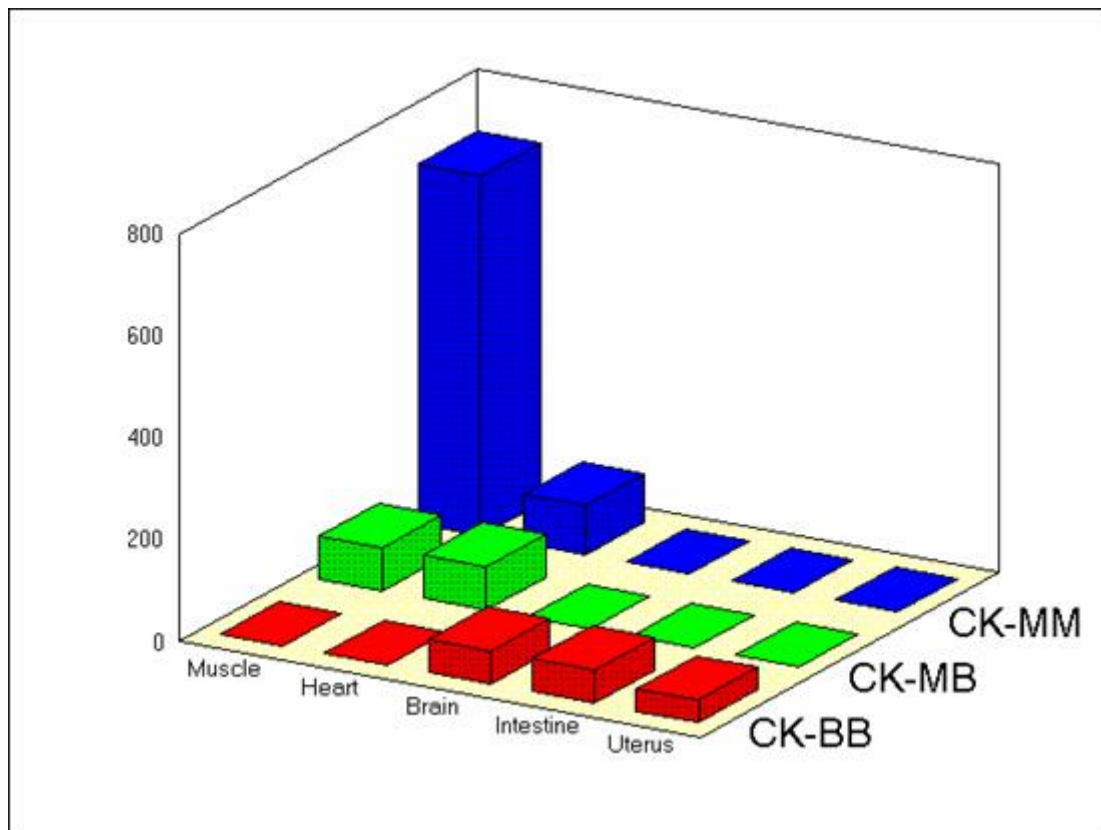


Figure 3: Distribution of CK activities in different organs of the human body [26]

2.2.2. Mb:

Mb is a small monomeric, respiratory hem protein consisting of a single polypeptide chain of about 153 amino acid residues which serves as intracellular oxygen storage site. [30]

The physiological function of the Mb is oxygen storage in the tissues until it is utilized during metabolism. It serves in some manners to aid the inflow of oxygen into cardiac and red skeletal muscle fibers. [31] When a muscle is exercised, it uses up available oxygen. Mb has oxygen bound to it, thus providing an extra reserve of oxygen so that the muscle can maintain a high level of activity for a longer period of time. The mitochondria of cardiac and red skeletal muscle myocytes do not lack for oxygen in normal steady states of sustained work. [32]

The role of Mb as an oxygen storage protein in the muscles of diving birds and mammals is well-established. [33] The Mb content is higher in more aerobic muscles and is highest in aerobic muscles with long contraction duration.

Changes in serum concentration of muscle proteins, such as CK and Mb, after exercise are studied to evaluate to use of such proteins as indicator of increased muscle membrane vulnerability. Mb in serum is a sensitive index for muscle damage, which rapidly appears in the circulation after exercise and reaches a peak hours earlier than CK. [34]

Plasma Mb is closely related to a quadriceps muscle cross-sectional area as a robust index of locomotor muscle mass, especially to the mass of Mb-containing fiber types that are crucial for daily performance. Functionally important aerobic slow-twitch type 1 (and 2a) fibers are the primary source of plasma Mb. [35]

Mb, the first biochemical marker to be commercially available for the diagnosis of acute myocardial infarction (AMI), is rapidly released from the areas of ischemic injury over a limited period. [36]

Mb is a sensitive but non-specific marker of AMI, as it is present in both skeletal and cardiac muscle, so its specificity is poor. Mb increases as soon as 1 to 2 hours after symptom onset in AMI. In emergency department patients with normal cardiac levels of TnI at presentation, a change in Mb provides a highly accurate diagnosis of AMI within 90 minutes. [37] It has been suggested that Mb is excellently suited for monitoring any form of acute muscle damage or increased muscle membrane permeability, be it after training or as a consequent of a disease or medication. [34] Angiogenesis, the process of formation of new blood vessels, can be therapeutic, as in endogenous response to arterial occlusions or when one seeks to deliver growth factors to increase blood flow and treat disorders of inadequate tissue perfusion. Nitric oxide (NO) plays a crucial role in both the endogenous angiogenic response to ischemia and in the therapeutic response sought after administration of growth factors. Hazarika, et al. [38] hypothesized that manipulating Mb expression in myocytes can alter NO availability and thus modulate angiogenesis after hind-limb ischemia in the setting of normal endothelial function. They concluded that increased Mb expression in myocytes attenuates angiogenesis after hind-limb ischemia by binding NO and reducing its bioavailability. Mb can modulate the angiogenic response to ischemia even in the setting of normal endothelium.

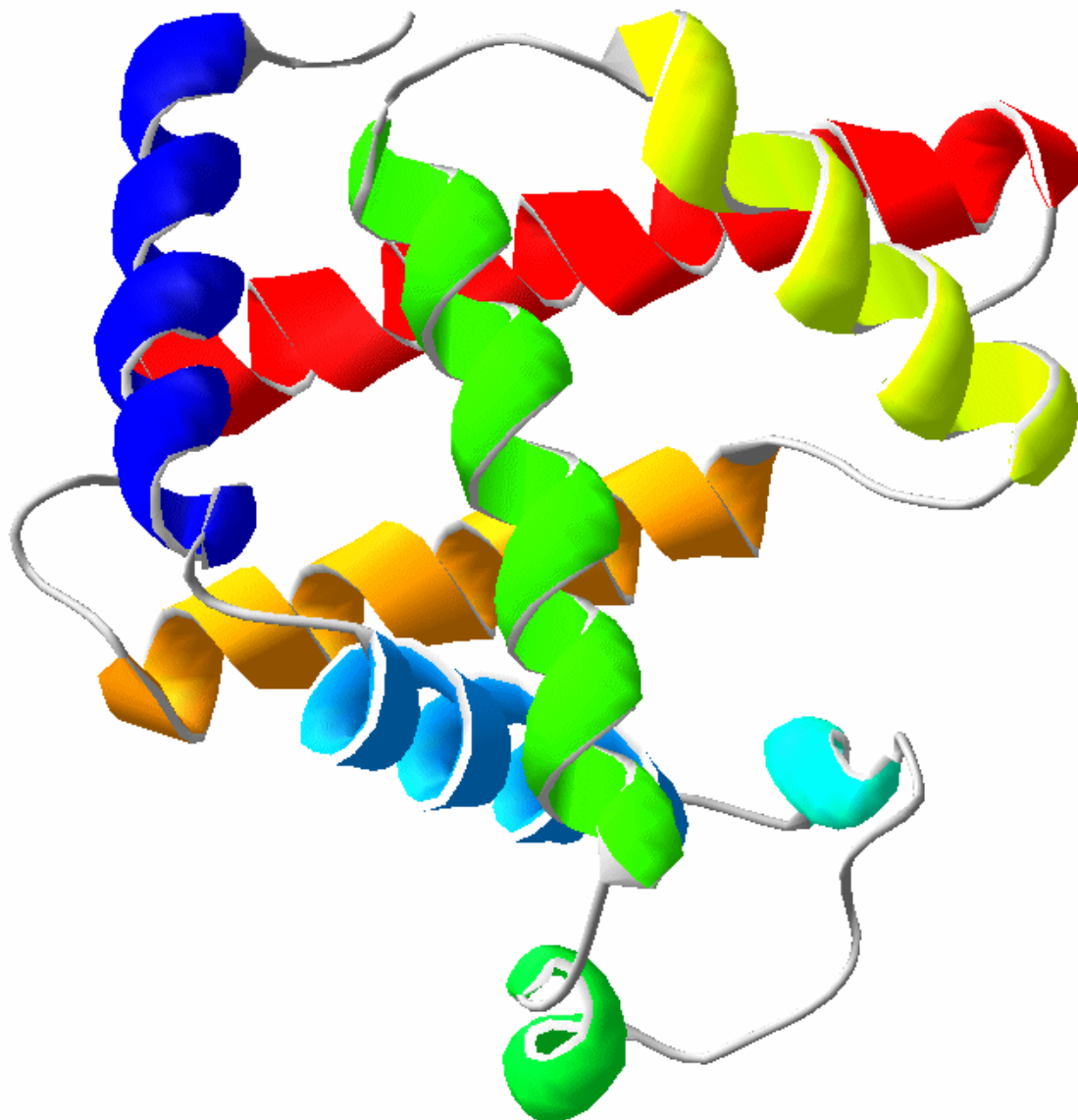


Figure 4: The single polypeptide chain of Mb (Reproduced from: <http://www.bio.davidson.edu/people/midorcas/animalphysiology/websites/2004/Goach/oxygen.htm>)

2.2.3. Fast myosin:

Myosin is considered to be the molecular motor that converts free energy derived from the hydrolysis of ATP into the mechanical work that drives muscle contraction. [39] Skeletal muscle myosin has a half-life as long as ~30 days. Myosin is composed of at least two low molecular weight protein chains of average

molecular weight 20,000-30,000 in addition to the two heavy or fibrous subunits of molecular weight 200,000. [40] Biochemical experiments on muscle contractile proteins have shown that, during the cross-bridge cycle, actin combines with myosin and ATP to produce force, ADP and inorganic phosphate.

Some of the contractile proteins have different isoforms depending on the type of fibre. Myosin, the major structural protein in muscle, [41] has different heavy and light isoforms depending on whether the fibre type is fast or slow. Myosin heavy chain (MHC) isoforms appear to represent the most appropriate markers for fibre type delineation. [42] On this basis, pure fibre types are characterized by the expression of single MHC isoform, whereas hybrid fibre type expresses two or more MHC isoforms. Fast myosin is characteristic of fast skeletal muscle only, while slow myosin is common to skeletal and cardiac muscle. [10]

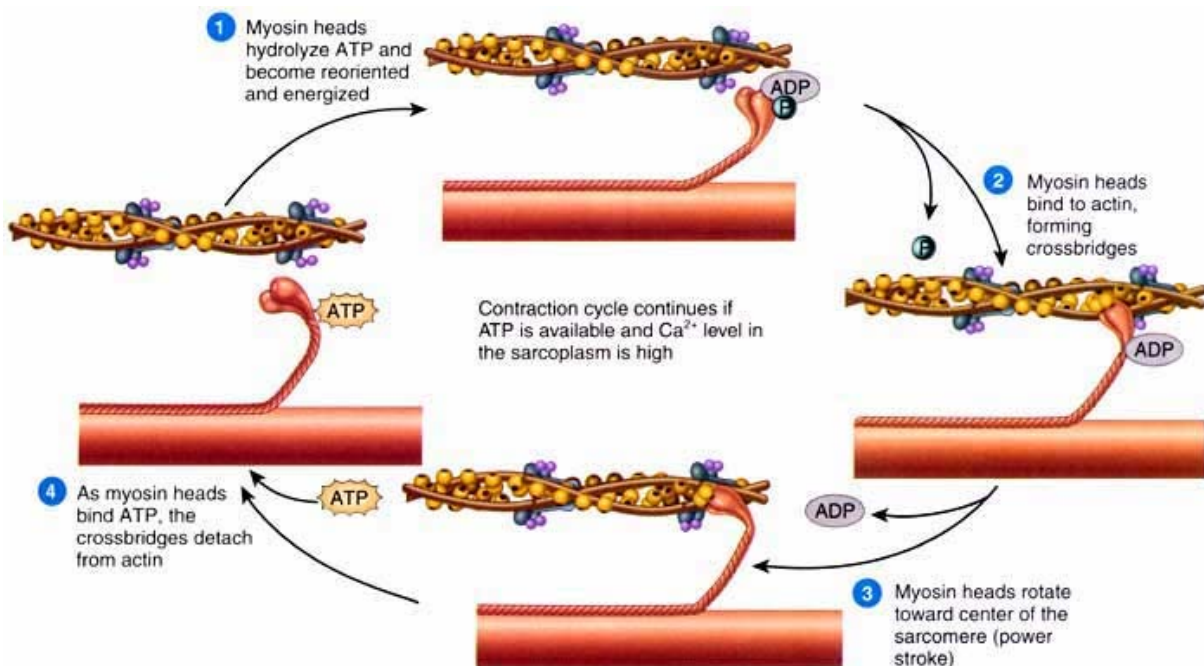


Figure 5: Diagram of interactions of myosin and actin in contraction cycle
(Reproduced from: <http://www.team-andro.com/phpBB3/bizepscurls-t163023-15.html>)

Human muscles are made up of a mixture of slow and fast fibres with approximately 50% of each. Although grade II and grade III lesions are detected by measuring of CK activity and both types of myosin, but fast myosin is a highly sensitive marker for grade I lesion. Fast myosin is a totally specific marker for

skeletal muscle, a property not shared by any of the markers currently in use such as CK and myoglobin (Mb). Myosin also has the advantage of being more sensitive and more stable in blood.

2.2.4. Alpha-actin:

Actin is a globular (G-actin), roughly 42-kDa protein found in eukaryotic cells. The thin actin filament is a dimeric polymer of G-actin sub-units arranged like two strings of beads twisted together.

In vertebrates, three main groups of actin isoforms, alpha, beta, and gamma have been identified. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins co-exist in most cell types as components of the cytoskeleton, and as mediators of internal cell motility.

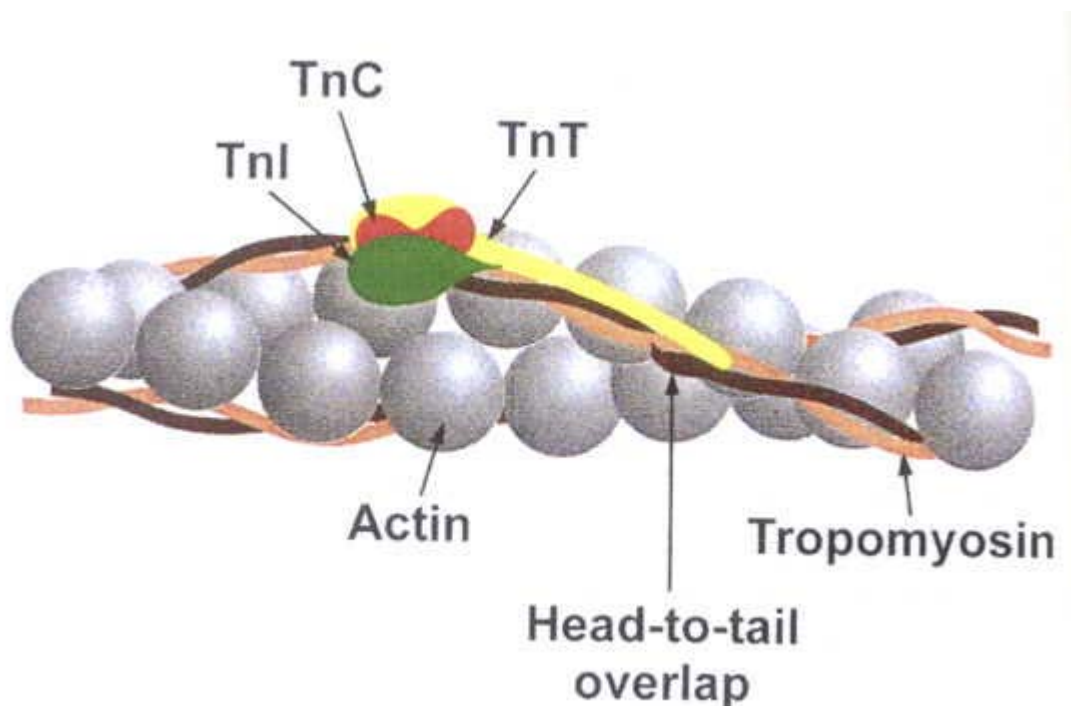


Figure 6: Diagram of actin filament (Reproduced from: www.mona.uwi.edu/fpas/courses/physiology/musc...)

Abundance of α -actin in the cell and its stability over time make it to be a good marker for detection of skeletal muscle damage alongside other currently used

biological markers. [23] The researchers suggested that skeletal α -actin may be a specific marker of muscle damage that could be measured when treating elite sportsmen and women.

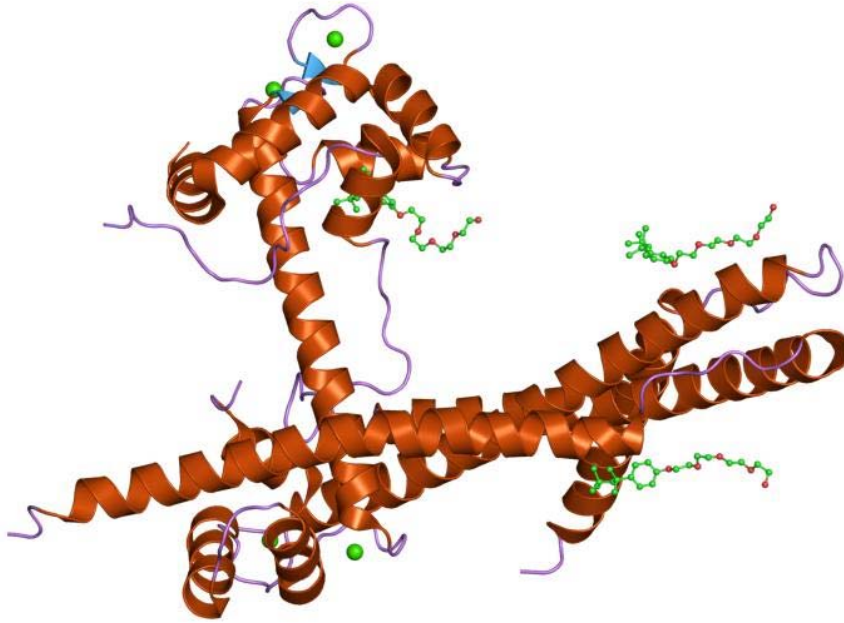
In another study, Martinez-Amatn, et al. [43] showed that α -actin is significantly increased in the serum of sportspeople with skeletal muscle damage. Their data showed that the serum concentration of α -actin is significantly higher in sportspeople with muscle damage (10.49 $\mu\text{g/ml}$) than in uninjured sportspeople (3.99 $\mu\text{g/ml}$). They suggested that the presence of circulating α -actin in the bloodstream may reflect cell lesions in patients with skeletal muscle damage. According to their new results, α -actin is a new and reliable marker of skeletal muscle damage in sportspeople which can be used for detection of muscle injury. Possible cross interference between skeletal and cardiac muscle damage can be discriminated by the combined use of α -actin and troponin I (TnI).

2.2.5. Skeletal troponin I:

Troponin I is the inhibitory subunit of troponin, the thin filament regulatory complex which confers calcium-sensitivity to striated muscle actomyosin ATPase activity. It binds to both actin and tropomyosin.

Troponin is found in both skeletal muscle and cardiac muscle, but the specific versions of troponin differ between types of muscle, due to the expression of different genes.

Although, detection of skeletal muscle injury is hampered by a lack of commercially assays for serum markers specific for skeletal muscle, serum concentrations of skeletal troponin I (sTnI) could meet this need. [44] The commonly used serum markers, e.g., lactate dehydrogenase, CK, and Mb lack specificity to skeletal tissue. MHC shows a delayed increase after exercise-induced muscle injury. [45] It is therefore not suitable for early diagnosis. TnI is a regulatory protein that is only expressed in striated muscle fibers. Marked increase in sTnI can be detected within 2-6 hours from the onset of exercise-induced muscle injury, with greater responses occurring for eccentric exercise.



A: Crystal structure of skeletal muscle troponin in the Ca²⁺-activated state



B: Crystal structure of skeletal muscle troponin in the Ca²⁺ free state

Figure 7: The crystal structure of skeletal muscle troponin (Reproduced from:

en.wikipedia.org/wiki/Template:PDB_Gallery/7125)

sTnI exists as 2 different isoforms, slow sTnI (ssTnI) and fast sTnI (fsTnI), produced in slow-twitch (ST) and fast-twitch (FT) fibers, respectively. Skeletal muscle injury led to the release of one or both isoforms of sTnI and their modified products into serum, activity-induced injury preferentially injured FT fibers. [44] In another study, Simpson and colleagues [46] concluded that changes in fsTnI and / or ssTnI could be influenced by clinical conditions, directly or indirectly, associated with rhabdomyolysis. Acute renal failure, compartmental syndrome, and cardiac abnormalities are common complications that can affect either the release or clearance of sTnI.

2.2.6. H-FABP:

At least 9 distinct types of specific proteins with tissue Specific distribution have been identified which can reversibly and non-covalently bind fatty acids, enhancing their aqueous solubility and facilitating their intracellular translocation. [47, 48]

The first, Mishkin and colleagues [49] demonstrated the binding of fatty acids to specific cytoplasmic proteins in various mammalian tissues. A possible physiologic role for these proteins in the transport and metabolism of fatty acids considered. This is particularly the case with regard to z protein which binds most of the oleic acid in liver supernatant in-vitro, they considered.

FABP, a small (15 kDa), water soluble, cytoplasmic protein is abundantly expressed in tissues with an active fatty acid metabolism like heart and liver. [50] Its concentration in the plasma of healthy persons is relatively low (<6 µg/L). [51] Its physiologic role is the transport of hydrophobic long chain fatty acids from the cell membrane to their intracellular sites of metabolism in the mitochondria, where they enter the citric acid cycle. [52] Thus FABPs may regulate cytoplasmic fatty acid concentrations and oxidative capacity. The capability of FABPs to bind retinoic acid and metabolites of the eicosanoid pathway suggests, moreover, their possible participation in cellular growth regulation and differentiation processes. There are two main types FABPs:

- Associated to plasma membrane (FABPpm)
- Intracellular or cytoplasmic FABPs(FABPc)

Chronic changes in cellular lipid metabolism are usually accompanied by concomitant adaptations in the tissue contents of both membrane associated and cytoplasmic FABPs, which relates to a common response also seen during regular physiological stimuli such as development, nutrition, and training.

Cytoplasmic FABPs are presently known to comprise a family of at least nine distinct types of FABP, with each type showing a characteristic pattern of tissue distribution. The cytoplasmic FABP content is related to the capacity to oxidize fatty acids. For striated muscles the content of heart-type FABP (H-FABP) was found to be related to the activity of the mitochondrial marker enzyme cytochrome c oxidase.

Among the members of the FABP family, H-FABP is most widely distributed within the organism. [53] H-FABP was first shown to be released from injured myocardium in 1988. H-FABP is abundantly expressed in cardiomyocytes, but to a lesser extent also in skeletal muscle, distal tubular cells of the kidney, specific parts of the brain, lactating mammary glands and placenta. [50] In comparison to Mb, because the cardiac tissue content of H-FABP is much higher than that of Mb and the normal plasma value of H-FABP is much lower than that of Mb, H-FABP is a more sensitive marker of myocardial injury than Mb.

The long-distance migratory flights of birds are predominantly fueled by the oxidation of fatty acids, which are sourced primarily from extracellular adipose stores. Pelters and colleagues [54] measured FABP in samples taken from the cardiac, pectoralis, and semimembranosus muscles of a long-distance avian migrant, the barnacle goose. This study has been the first time that FABP has been quantified in the locomotor muscles of a bird that is capable of migrating long distance. The FABP content in the adult barnacle goose skeletal muscles have been similar to those have found in the skeletal muscles of higher vertebrates such as rats and humans. However, the FABP content of the cardiac muscle of barnacle goose has been markedly lower than that of rats or humans, the physical demands for migrating geese are at least as high as those of running mammals. In Table 1, the FABP content of cardiac and skeletal muscles of human, rat and goose has been compared. Vork and colleagues [55] in their study, an ELISA assay for the determination of H-FABP in rat muscles, observed a positive relationship between the level of H-FABP and the percentage of fiber type I.

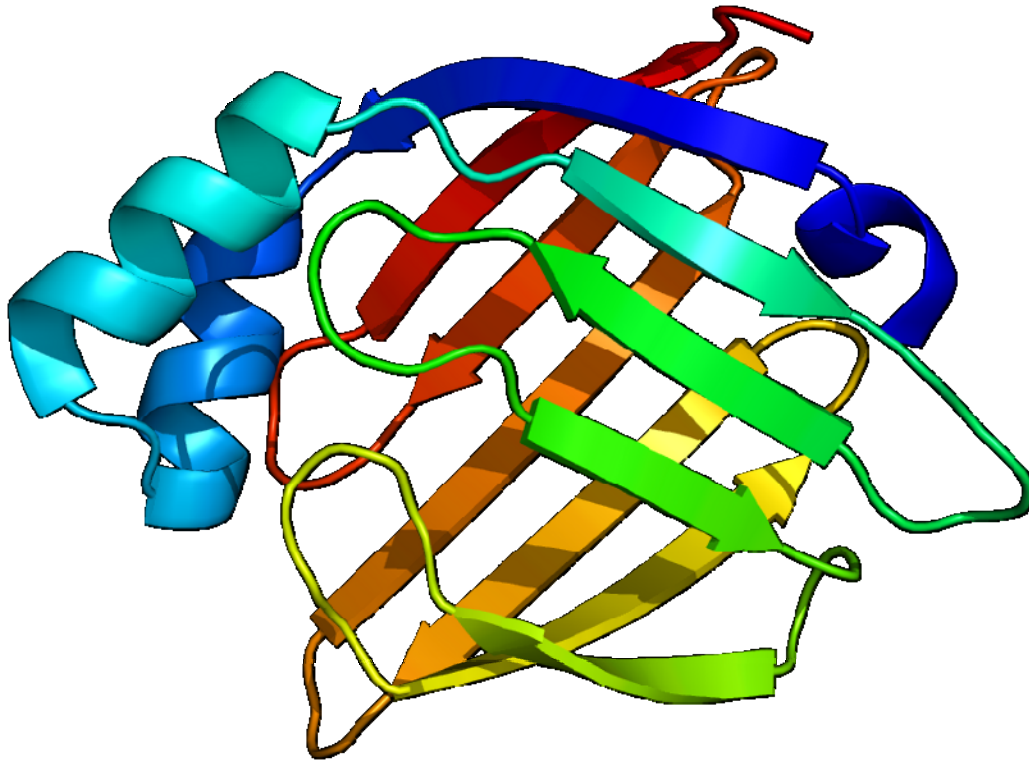


Figure 8: Diagram of FABP (Reproduced from: en.wikipedia.org/wiki/FABP3)

Table 1: The FABP content of cardiac and skeletal muscles of goose, rat and human ($\mu\text{g/g}$ wet wt)

Muscle	Cardiac muscle	Skeletal muscles	Reference
Goose	60	10-100	Pelsers et al., 1999
Rat	740 \pm 120	13-303	Vork et al., 1991
Human	520 \pm 60	40-140	Nieuwenhoven et al., [56] 1995

FABP is one of the novel and promising plasma markers for detection of tissue injury. [50] H-FABP became recognized as a cardiac marker in the 1990s. [57]

H-FABP appears as early as 1.5 hours after onset of acute myocardial infarction and is largely excreted from the kidney and eliminated from the blood in 24-36 hours. [58] Tanaka, et al. [58] reported that the serum and urine samples obtained of AMI patients only 1.5 hours after the onset of symptoms showed elevated H-FABP levels, while in the same serum samples the activity of the myocardial-specific isoenzyme of CK (CK-MB) was still normal. Monique J. M. de Groot and colleagues [59] showed that using individually estimated clearance rates for H-FABP and Mb, reliable estimates of myocardial infarct size can be obtained within 24 hours, while for the established markers CK or α -hydroxybutyrate dehydrogenase (HBDH), a time span of 72 hours is required.

The severity of pulmonary embolism (PE) ranges from asymptomatic to cardiogenic shock. [60] H-FABP measured on admission in patients with confirmed acute pulmonary embolism (APE) is useful for short-term risk stratification. It appears to be superior to cardiac troponin I (cTnT), N-terminal pro brain natriuretic peptide (NT-proBNP) and Mb in the prediction of 30-day APE-related mortality. [61]

H-FABP appears to be a valid serum biomarker for the early diagnosis of stroke. [62] H-FABP, troponin-I (TnI), and CK-MB concentrations allowed a correct discrimination between AMI (increase of all three markers) and stroke (increase of H-FABP with normal TnI and CK-MB). Wunderlich [63] showed for the first time quantitative data of serum brain-type (B-) FABP and H-FABP being elevated early in acute ischaemic stroke.

Exercise and physical training have been known to damage the skeletal muscles. For the first time Sorichter [64] showed that plasma FABP increases after physical exercise by healthy subjects and that its pattern of release into and clearance from the blood is similar to that of Mb. In another study the plasma FABP and CK activity of a group of junior rowers were measured for 5 weeks. [65] The researchers concluded that although both parameters can be used to detect exercise-induced muscle damage early, it is speculated that FABP measurement will be more important for monitoring acute muscle damage among elite athletes after a single training session. The baseline CK is a better indicator for the chronic effect of training conducted on the previous 2 days of exercise training. Malek, et al. [66] investigated whether H-FABP plasma concentration increases in healthy

subjects after exercise. They observed that the H-FABP concentration increased on exercise in healthy subjects independently of the time of the run, time-to-test and hours of training per week.

2.2.7. Mb/FABP ratio:

Mb and FABP each are useful as early biochemical markers of muscle injury. Nieuwenhoven and colleagues [67] evaluated the plasma ratio of Mb over FABP to distinguish myocardial from skeletal muscle damage. In human heart tissue, the Mb/FABP ratio is 4.5 and in skeletal muscles varies from 21 to 73. In Table 2 the ratio of Mb over FABP has been compared in cardiac and skeletal muscle and also in serum and plasma of patients.

Table 2: The ratio of Mb over FABP in heart, skeletal muscles, plasma and serum of patients

Heart *	Various skeletal muscles	Plasma **	Serum ***
4.5 ± 0.8	21-73	4.4 ± 1.4	45 ± 22

* In intact human heart, obtained from autopsy

** Over 24 h after AMI

*** In patients who underwent aortic surgery, between 12 to 24 h after surgery

Both Mb and FABP in plasma can be used as markers of loss of cardiac and /or skeletal muscle cell integrity. Both proteins show a similar pattern of release into and clearance from plasma. The ratio of the plasma or serum concentrations of Mb over FABP reflects the ratio in which the proteins occur in the injured tissue. Hence, measurement of Mb and FABP in the same blood sample and expression of their ratio is useful to determine the origin of the proteins that are released into the vascular compartment. In this way, one can discriminate between damage inflicted upon cardiac and skeletal muscle tissue.

Both plasma FABP and Mb increase after physical exercise by healthy subjects. For both FABP and Mb a significant increase reaches earlier (30 minutes) than for CK (2 hours), indicating the usefulness of the former for the early detection of muscle injury. [64] In addition, both FABP and Mb return to baseline values at 24 hours after exercise whereas CK remains elevated until (at least) 48 hours after exercise. The relatively short half-life of FABP and Mb in plasma relates to their rapid elimination from plasma mostly by renal clearance. FABP and Mb, in comparison with CK, are also more useful for the separate monitoring of muscle injury during repeated exercise bouts.

The Mb to H-FABP ratio seems to be useful to identify the type of muscle injured. [68] New studies are necessary to evaluate its diagnostic accuracy.

2.3. Measuring and Values:

Based on assessment of Mb and H-FABP in practical part of the current study, we reviewed the laboratory methods for measuring of these two markers.

2.3.1. Myoglobin (Mb):

Mb has been measured by the various different immunological methods. Mikhail A. Grachev, et al. [69] used a radio immuno assay (RIA) method. In that procedure yields had been resulted in 50-60 min after the arrival of the specimen. Thomas Olsson [70] and colleagues in their study determined Mb in the range of 10-500 µg/l by the chemiluminescent luminal reaction. Only 50 µl of serum has been needed. However, the chemiluminescent luminal reaction is not specific for Mb since also other haemoproteins, e.g., haemoglobin (Hb) will catalyze the luminescent agglutination tests for the rapid detection of elevated levels of serum Mb were studied by Konings, et al. [71] They concluded that Mb agglutination test could be of value in the exclusion of myocardial infarction. Nishida and colleagues [72] developed a sensitive sandwich enzyme immunoassay (EIA) for human Mb. In their study the mean normal serum concentration of Mb has been 21.5 ± 6.0 µg/l (mean \pm SD) in males (range, 10.5-33) and 16.9 ± 5.8 µg/l in females (range, 8-31). Mb in the serum is not influenced by storage for a long time or by repeated freezing and thawing sample to repeat assays. [73] An automated nephelometric immunoassay involving shell/core particles for determination of Mb (Behringwerke)

was evaluated with the BNA Nephelometer by Delanghe, et al. [74] In that time immunonephelometric determination of serum Mb has been a fast, convenient and reliable method appropriate to the emergency laboratory. A turbidimetric immunoassay using shell/core particles for determination of myoglobin was evaluated of the Behring Turbitimetr analyzer. [75] Values obtained by turbidimetry have been comparable to those obtained by the latex agglutination test (Behringwerke). Silva, et al. [76] produced monoclonal antibodies useful for the rapid measurement of Mb in a one-step, two-site particle concentration fluorescence immunoassay (PCFIA). This method is specific for Mb, has good analytical precision, and provides results in ~1 h quite comparable with those found by RIA. Yoshinori Uji and colleagues [77] described the evaluation of Mb by a fully automated immunoturbidimetric quantitation by a latex agglutination technique. Serum Mb levels in males have been markedly higher than those in females that may be explained by the greater muscle mass in males than females. Mario Plebani and Martina Zaninotto [36] evaluated a new method for Mb determination using an electrochemical immunosensor that allows the sensitive measurement of the marker in less than 15 min. In recent years the useful laboratory methods have been developed. Matveeva and colleagues [78] utilized enhanced fluorescence on silver island films (SIFs) to develop a sandwich-format immunoassay for the cardiac marker Mb. The initial results for the sandwich Mb immunoassay (on SIF-modified surface using wet chemistry) has showed that it is possible to detect Mb concentrations below 50 ng/ml, which is lower than clinical cut-off for Mb in healthy patients. Recent work has demonstrated an approach to independently measure oxygen saturation of Hb and Mb in optical spectra collected in vivo. Marcinek et al. [79] described a strategy to quantify the contributions of Hb and Mb to in vivo optical spectra. Biochemical analysis of muscle homogenates confirmed that the wavelength shift of the combined Hb/Mb peak in in vivo spectra reflects the ratio of concentrations (Hb/Mb) in muscle. They provided a fully noninvasive approach to measuring local respiration that can be adapted for clinical use. Mion and colleagues [80] evaluated the analytical and clinical performance of the Evidence Cardiac Panel. The Evidence Cardiac Panel, an automated protein biochip microarray, allows the simultaneous determination of creatine kinase MB (CK-MB), Mb, glycogen phosphorylase BB (GPBB), H-FABP, carbonic anhydrase III (CA III), cardiac troponin I (cTnI).

2.3.2. H-FABP:

An enzyme linked immuno-sorbent assay (ELISA) of the sandwich type for the determination of H-FABP was developed by Vork, et al. [55] making use of the streptavidin-biotin system. In their study the FABP content of adult rat heart muscle was found to be 0.740 ± 0.034 mg/g wet weight. The FABP content of a number of skeletal muscles varied from 0.023 to 0.303 mg/g wet weight and was related to the content of type 1 muscle fibers of these tissues. Crisman, et al. [81] showed H-FABP is immunologically distinguishable from the hepatic FABP and heart Mb. It appears that H-FABP, like Mb, is an abundant soluble protein found in the cytosol. Yasuhiko Ohkaru, et al. [82] developed a sandwich enzyme-linked immunosorbent assay (ELISA) for the determination of human heart type fatty acid-binding protein (HH-FABP) in human plasma and urine using the combination of two distinct monoclonal antibodies (MAbs) directed against human H-FABP purified from human heart muscle. The minimum detection limit of the ELISA has been 1.25 ng/ml. The normal mean (\pm SD) level of human H-FABP has been 3.65 ± 1.81 ng/ml, and that in urine has been 3.20 ± 2.70 ng/ml. W. Roos, et al. [83] described the development and characterization of monoclonal antibodies to human H-FABP, illustrating the value of BIAcore biosensor methodology as an adjuvant to hybridoma technology. They tested the cross-reactivity of other types of cytoplasmic FABP by measuring the binding of human intestinal, human liver and bovine heart FABP to the monoclonal antibodies. To allow a more rapid determination of heart-type FABP concentration in plasma a direct non-competitive (sandwich-type) ELISA was developed by Wodzig, et al. [84] which uses high-affinity monoclonal antibodies to FABP. Total performance time of the one-step immunoassay is 45 minutes. The serum FABP concentration measured in 79 healthy individuals has been 1.6 (0.8) [mean (SD), range 0.3-5.0] μ g/L. Robers, et al. [85] found that the simplicity, reproducibility, and full automation in a widely used clinical chemistry analyzer like the COBAS MIRA seem to be factors of choice for the FABP latex immunoassay for routine clinical diagnosis of AMI. A rapid lateral-flow assay for detection of H-FABP was described by Chan and colleagues [86]. They found a good correlation between the conventional ELISA and the newly developed lateral-flow assay. The FABP lateral-flow assay can be performed at the site of patient care delivery such as emergency department (ED). It is a quantitative test using a small quantity of sample and returns results within 5

min. Van der Voort and colleagues [87] studied a displacement immunosensor for continuous measurement of FABP. Such continuous measurement would not only be useful for patients entering the ED with chest pain, but may also be applied for non-invasive detection of coronary reperfusion. Early detection of tissue injury is in general important in stratifying patient treatment and reducing mortality and morbidity. Rapid release from injured cells and appearance in plasma, specific and high tissue content and low plasma level in normal healthy controls are the characteristics of an ideal biochemical marker. A promising marker for early detection of tissue injury is FABP. To take full advantage of the characteristics of the early marker FABP, the development of a rapid detection method is crucial. So in another study, Chan and colleagues [88] reviewed the development of immunoassay for the quantification of FABP in buffer, plasma or whole blood, from 1997 till 2003. They concluded the application of FABP as an early tissue injury marker has a great potential for many clinical purpose. Zaninotto, et al. [89] evaluated the analytical characteristics of a new quantitative and fully automated H-FABP assay (Randox Laboratories Ltd., Crumlin, UK) and compared its clinical performance with respect to the Mb assay (Dade Behring, Milan, Italy). The best cut-off and the associated diagnostic efficacy have been 95 µg/L and 89.47% for Mb and 5.09 µg/L and 98.70% for H-FABP, respectively. The H-FABP assay evaluated is part of a biochemical panel, the Evidence Cardiac Panel (Randox Laboratories Ltd., Crumlin, UK), which allows quantitative fully automated simultaneous determination of six markers: creatine kinase MB, Mb, glycogen phosphorylase BB, H-FABP, carbonic anhydrase III and cardiac troponin I. Sample pre-treatment is not necessary, and the time required to process sample and obtain all cardiac panel results, as well as the single biochemical marker value, is approximately 80 min.

2.4. Anaerobic Threshold:

For a long time maximal oxygen uptake as considered to be the best means of determining endurance capacity. [90, 91] This is, however, only a valid measurement for short-term endurance work loads. A better measure to ascertain middle-term and long-term endurance has proven to be the determination of the aerobic-anaerobic threshold. Lactate is produced in skeletal muscle as a direct result of increased metabolic rate and glycolytic carbon flow. [92] The blood lactate

concentration is the result of its production in muscles and appearance in the blood and its removal from the blood and catabolization. The rise in lactic acid concentration to 4 mmol/l in peripheral blood during gradual increases in workloads can be considered as the criteria for the establishment of the aerobic-anaerobic threshold in spiroergometric testing. As Cunha and colleagues [93] stated the anaerobic threshold (AT) can be defined as the exercise intensity above which the blood lactate concentration increases over proportionally due to an augment in the anaerobic contribution for ATP re-synthesis. Experimental evidence indicates a threshold representing a balance between removal and release of lactate from and into the plasma compartment. [94] Considering the AT at the fixed value of 4 mmol/l lactate does not take into account the individual kinetics of the blood lactate concentration curve. Therefore, the individual AT can be located above or below the 4 mmol/l blood lactate level. Cordova and colleagues [95] used an incremental cycle ergometer to determine the AT defined to them as a blood lactate concentration of 3.5 mmol/l. In their study, to determine AT, each participant underwent a cycle ergometer test consisting of two 5 minutes exercise periods at progressive intensities with a 10 minutes rest between the two rounds. Capillary blood samples have been collected at rest both before exercise and at the end of each stage at the 1st, 3rd, and 5th minutes of the rest interval. Blood lactate concentrations have been determined electroenzymatically and the exercise load corresponding to the 3.5 mmol/l lactate threshold has been determined by means of linear interpolation. To understand the concept of AT, it is important to understand the metabolic systems that provide energy during exercise. [96]

2.5. Exercise Metabolism:

The ability of human to exercise depends on the conversion of chemical energy to mechanical energy in skeletal muscle. The source of chemical energy in muscle is ATP. The store of ATP in muscle is small and would be quickly consumed in contraction muscle if it were not immediately replenished, as the demand for ATP can increase more than 100-fold in the transition from rest to maximal exercise. During most exercise, the ATP demand and provision are precisely matched and the store of ATP in the muscle is maintained. Therefore, the metabolic pathways

that provide ATP within contracting skeletal muscle rapidly respond to the increased need for ATP during exercise. [97]

At rest the energy that the body needs is derived almost equally from the breakdown of carbohydrates and fats. Proteins serve important functions as enzymes that aid chemical reactions and as structural building blocks, but usually provide little energy for metabolism. During intense, short-duration muscular effort, more carbohydrate is used, with less reliance on fat to generate ATP. Longer, less intense exercise utilizes carbohydrate and fat for sustained energy production. [98] The amount of carbohydrate utilized during exercise is related to both the carbohydrate availability and the muscles' well-developed system for carbohydrate metabolism. Fat provides a large portion of the energy during prolonged, less intense exercise.

To generate ATP, a phosphate group is added to the relatively low-energy compound, ADP, a process called phosphorylation. Some ATP is generated independent of oxygen availability, and such metabolism is called substrate-level phosphorylation. Some other ATP-producing reactions occur without oxygen, a process called anaerobic metabolism. When these reactions occur with the aid of oxygen, the overall process is called aerobic metabolism, and the aerobic conversion of ADP to ATP is oxidative phosphorylation.

Cells generate ATP through three different processes or systems:

- The ATP-PCr system (Immediate energy)
- The lactic acid system (Short-term energy)
- The aerobic system (Long-term energy)

The ATP-PCr system (immediate energy) is the quickest source of ATP for muscle actions. Performances of short duration and high intensity such as 100-m sprint, 25-m swim, smashing a tennis ball during the serve, or thrusting a heavy weight upwards require an immediate and rapid energy supply. The high energy phosphates ATP and PCr stored within muscles almost exclusively provide this energy. The term phosphagens identifies these intramuscular energy surces. [99] Each kilogram of skeletal muscle stores approximately 5 millimoles (mmol) of ATP and 15 mmol of PCr. The quantity of intramuscular phosphagens significantly influences ability to generate all out energy for brief duration. The enzyme CK,

which triggers PCr hydrolysis to resynthesize ATP, regulates the rate of phosphagen breakdown.

The lactic acid system (Short-term energy) does not produce large amounts of ATP. The intramuscular phosphagens must continually resynthesize rapidly for strenuous exercise to continue beyond a brief period. During intense exercise, intramuscular stored glycogen provides the energy source to phosphorylate ADP during anaerobic glycolysis. Without adequate oxygen, pyruvate converts to lactate. Anaerobic energy for ATP resynthesis from glycolysis can be viewed as reserve fuel that activates when the oxygen demand to oxygen utilization ratio exceeds 1.0. Anaerobic ATP production remains crucial during a 440-m run, 100-m swim, or in multiple-sprint sports like ice hockey, field hockey, and soccer. These activities require rapid energy transfer that exceeds that supplied by stored phosphagens. Despite limitation of low amount ATP production, the combined actions of the ATP-PCr and glycolytic systems allow the muscles to generate force even when the oxygen supply is limited. These two systems predominate during the early minutes of high-intensity exercise.

Aerobic metabolic reactions (Long-term energy) provide for the greatest portion of energy transfer, particularly when exercise duration extends longer than 2 to 3 minutes. ATP is provided from food mainly sugar and fat. This system is the prime energy source during endurance activities. Oxygen-consuming reactions supply the energy for steady-rate exercise, any lactate produced either oxidized or reconverts to glucose in the liver, kidneys, and skeletal muscles. No accumulation of blood lactate occurs under these metabolic conditions. Some conditions may affect the capacity of aerobic exercise. Patients with metabolic syndrome have lowered aerobic exercise capacity, which might be due to impaired skeletal muscle mitochondrial oxidative phosphorylation and dysregulation of intramyocellular fatty acid metabolism. [100]

3. Methodology

Firstly a systematic database search of PubMed, Web of Scholar Google, Web of Library of Bielefeld University and also the “Interlibrary Loan” via the later library, and hand searching the reference lists of these retrieved articles was performed to achieve the potential muscle injury markers. Key words searched were “muscle injury markers”, “skeletal muscle injury”, “muscle injuries”, and “sport injuries”.

3.1. Muscle injury markers:

Some of muscle injury markers were reviewed, as discussed in chapter 2, and have been mentioned below:

3.1.1. CK:

This enzyme was found mainly in muscular, electric and nervous organs. [101]

In veterinary medicine, serum creatine-kinase (CK) activity is mostly used to assess skeletal muscle damage. [102]

3.1.2. Mb:

Mb is a compact, highly soluble, and typical globular protein and is found in many animal cells. [103, 104] Like haemoglobin, it combines reversibly with molecular oxygen. Mb in serum is a sensitive index for muscle damage.

3.1.3. Fast myosin:

In mammals, slow/type I fibers express β -MHC, while fast/type II fibers express one or more of the six type II skeletal muscle myosin genes. [105] Human skeletal muscle fibers express five highly conserved type-II MHC genes. Fast myosin is a totally specific marker for skeletal muscle.

3.1.4. α -actin:

Cell division, cell motility and the formation and maintenance of specialized structures in differentiated cells depend directly on the regulated dynamics of actin cytoskeleton. [106] α -actin is a new and reliable marker of skeletal muscle damage in sportspeople.

3.1.5. sTnl:

sTnl has been identified as a potential marker of skeletal muscle injury in humans and animals. [107] Tnl is a regulatory protein that is only expressed in striated muscle fibers.

3.1.6. H-FABP:

Nine types of cytoplasmic FABP have been well identified up to now. [108] Among the members of the FABP family, H-FABP is most widely distributed within the organism. H-FABP appears to be a valid serum biomarker for the early diagnosis of: AML, stroke, and acute muscle damage.

3.1.7. Mb/FABP ratio:

Both plasma FABP and Mb increase after physical exercise by healthy subjects. Since the ratio of Mb over H-FABP differs between heart and skeletal muscles, it can be used to differentiate myocardial injury from skeletal muscle injury. It also seems to be useful to identify the type of muscle injured.

In practical part of the current study, we evaluated the effects of low and high intensity exercises on serum concentrations of Mb and H-FABP in well-trained athletes.

3.2. *Participants:*

We invited the students of sport science through bulletins and also in their classes. Five well-trained athletes were enrolled in this study. We explained the risks and benefits of study to all participants, 5 well-trained athletes, mean age 36.1 ± 9.98 , and informed consent letters were obtained from them. All tests were performed in a laboratory (temperature $19-22^{\circ}\text{C}$, humidity 40-60%) on a motor driven treadmill (model Ergo ELG2, Woodway, Germany).

3.3. Determining of anaerobic threshold:

The anaerobic threshold (AT) can be defined as the exercise intensity above which the blood lactate concentration increases over proportionally due to an augment in the anaerobic contribution for ATP re-synthesis. [93]

We determined the AT via graded running on a treadmill with increased velocity at regular intervals (the velocity was increased 0.4 m/s every 5 minutes). The heart rate was controlled regularly and blood samples were taken in small amounts from the ear of participants at each increment to determine the lactate concentration with an automatic analyser (SUPER GL, Dr. Müller Gerätebau GmbH). According to Heck and colleagues [91] AT is as the running velocity when lactate concentration reaches 4 mmol/l.

3.4. Prolonged constant-load exercise bouts:

After the determination of AT, participants were scheduled to complete two 30-minutes exercise bouts.

3.4.1. Low intensity/horizontal (LH):

The first constant-load exercise bout consists of 30 minutes of running on treadmill at a velocity of 70% of AT and an elevation of 1% [LH: 70%AT, +1%].



3.4.2. High intensity/downhill (HD):

At the second constant-load exercise bout the participants were asked to run for 30 minutes downhill on the treadmill at a velocity of 90% of AT and 10% declination [HD: 90%AT, -10%]. This step was started by warming up of subjects on a treadmill at a velocity of almost 55% of AT for 3 minutes.

All these three steps of the test (determination of AT and two prolonged constant-load exercise bouts) were performed in three weeks, subsequently for each subject.



3.5. Blood sampling and markers measuring:

Capillary blood samples for the determination of H-FABP and Mb were collected from a superficial forearm vein in EDTA coated tubes, before the start of the test and again one hour after running on the treadmill at each constant-load exercise bout.

According to the instruction of relevant kits, blood samples for determining Mb and H-FABP were centrifuged after 20 minutes and after 2 hours, respectively. Plasma samples were frozen and stored at -20°C until assayed.

H-FABP and Mb were measured by quantitative tests based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA) [H-FABP ELISA, Myoglobin ELISA, DRG Instruments GmbH, Germany].

3.5.1. Instructions for Mb measuring:

- 1- The plasma samples were diluted 10 fold before use. A series of 2 ml micro centrifuge tubes were prepared and mixed 20 μ l of plasma sample with 180 μ l of sample diluent.
- 2- 20 μ l of Mb standards, diluted specimens and diluted controls were dispensed into the appropriate coated wells in the holder.
- 3- 200 μ l of enzyme conjugate reagent was dispensed into each well and thoroughly mixed for 30 seconds and incubated at room temperature (18-25°C) for 45 minutes.
- 4- The incubation mixture was removed by flicking plate contents into a waste container.
- 5- The microwells were rinsed and flicked 5 times with deionized water.
- 6- The wells were strucked sharply onto absorbent paper to remove all residual water drops.
- 7- 100 μ l of TMB (tetramethyl-benzidine) reagent solution was dispensed into each well and gently mixed for 5 seconds and incubated at room temperature for 20 minutes.
- 8- The reaction was stopped by adding 100 μ l of stop solution to each well and gently mixed 30 seconds.
- 9- The absorbance was read at 450 nm with a microtiter well reader within 15 minutes.

3.5.2. Instructions for H-FABP measuring:

- 1- The plasma samples were diluted 20 fold prior to use. A series of 2 ml micro centrifuge tubes were prepared and mixed 15 μ l of plasma sample with 285 μ l of sample diluent.
- 2- 100 μ l of standard, diluted specimens, and diluted controls were dispensed into appropriate coated wells in the holder and incubated on orbital micro plate shaker at 750 rpm at room temperature (18-25°C) for 60 minutes.

- 3- The incubation mixture was removed by flicking plate contents into an appropriate Bio-waste container.
- 4- The microtiter wells were rinsed and flicked 5 times with 300 µl working wash buffer.
- 5- The wells were strucked sharply onto absorbent paper to remove all residual water droplets.
- 6- 100 µl of enzyme conjugate reagent was dispensed into each well and incubated on orbital micro-plate shaker at 750 rpm at room temperature (18-25°C) for 60 minutes.
- 7- The incubation mixture was removed by flicking plate contents into an appropriate Bio-waste container.
- 8- The microtiter wells were rinsed and flicked 5 times with 300 µl working wash buffer.
- 9- The wells were strucked sharply onto absorbent paper to remove all residual water droplets.
- 10- 100 µl of TMB (tetramethyl-benzidine) reagent was dispensed into each well and gently mixed for 10 seconds and incubated at on orbital micro-plate shaker in the dark for 20 minutes.
- 11- The reaction was stopped by adding 100 µl of stop solution to each well. The optical density was read at 450 nm with a microtiter plate reader within 15 minutes.

3.6. Statistical analysis:

The mean and standard deviation of H-FABP and Mb were computed. The non-parametric of Mann-Whitney U test for dependent variables was performed with SPSS version 17.0 to test for significant differences between the values of H-FABP and Mb or the ratio of these markers, immediately before and 1 hour after running on treadmill in both steps of current study. $P < 0.05$ (*) was considered to indicate statistical significance.

4. Results

4.1. *Characteristic issues of muscle injury markers:*

As mentioned above, in the current study, firstly we reviewed some of muscle injury markers, as were explained in section 2. Their characteristic issues have been summarized below:

4.1.1. CK:

- High CK activity is detectable in muscle, heart and brain.
- CK activity is also detectable in serum.
- CK-MM is the main isoenzyme in muscle.
- CK is the most common serum marker for skeletal muscle injury, but CK is not an ideal marker for several reasons:
 - Lack of tissue specificity
 - Inability to reveal damage to specific skeletal fiber types
 - In low concentration of glutathione, CK has low value.

4.1.2. Mb:

- Mb is a small monomeric, respiratory hem protein.
- Aerobic slow-twitch type 1 (and 2a) fibers are the primary source of plasma Mb.
- It has been suggested that Mb is an excellent marker of acute muscle damage or increased muscle membrane permeability.
- Mb is a sensitive but non-specific marker of AMI.
- Mb can modulate the angiogenic response to ischemia.

4.1.3. Fast myosin:

- Myosin is the major structural protein in muscle.

- Myosin is more sensitive and more stable marker than CK and Mb in blood.
- Fast myosin is a totally specific marker for skeletal muscle.
- MHC shows a delayed increase after exercise-induced muscle injury. It is therefore not suitable for early diagnosis.

4.1.4. α -actin:

- Actin is a globular protein found in eukaryotic cells.
- α -actins are found in muscle tissues.
- α -actins are a major constituent of the contractile apparatus.
- α -actin is abundant in the cell.
- α -actin is a new and reliable marker of skeletal muscle damage.
- Discrimination between skeletal and cardiac muscle damage can be done by the combined use of α -actin and Tnl.

4.1.5. sTnl:

- Tnl is the inhibitory subunit of troponin.
- Tnl is only expressed in striated muscle fibers.
- ssTnl is produced in slow-twitch (ST) fibers.
- fsTnl is produced in fast-twitch (FT) fibers.
- Activity-induced injury preferentially injured FT fibers.
- The release or clearance of sTnl can be affected by some conditions such as renal failure, compartment syndrome, and cardiac abnormalities.

4.1.6. H-FABP:

- FABP can reversibly and non-covalently bind fatty acids and transport them from the cell membrane to mitochondria.
- FABP is one of the novel and promising plasma markers for detection of tissue injury.
- Among the members of the FABP family, H-FABP is most widely distributed within the organism.
- H-FABP is abundantly expressed in cardiomyocytes, but to a lesser extent in skeletal muscles and other tissues with an active fatty acid metabolism.
- H-FABP appears to be a valid serum biomarker for the early diagnosis of: AMI, stroke, and acute muscle damage.

4.1.7. Mb/H-FABP ratio:

- Discrimination between myocardial and skeletal muscle injury.
- The Mb to H-FABP ratio seems to be useful to identify the type of muscle injured.

4.2. The results of practical part of project:

Our study in practical part comprised 5 well trained athletes who performed all steps of test, completely. Firstly, they run on a treadmill with increased velocity at regular intervals (the velocity was increased 0.4 m/s every 5 minutes). The heart rate was controlled regularly and blood samples were taken in small amounts from the ear of participants at each increment to determine the lactate concentration with an automatic analyser. We determined their AT via graded running on a treadmill in this step.

Table 3: Heart rate and blood lactate concentration of participants on graded running for determining of AT

Velocity (m/s)	Time (min)	Heart rate (bpm)					Lactate concentration (mg/dl)				
		S-1	S-2	S-3	S-4	S-5	S-1	S-2	S-3	S-4	S-5
1.6	5	100	102	113	102	98	0.92	0.91	1.16	0.95	0.84
2.0	5	104	129	127	110	127	0.74	1.69	1.33	1.12	0.95
2.4	5	121	132	130	122	139	0.88	2.16	1.33	1.25	1.35
2.8	5	132	150	146	129	151	1.28	2.84	2.35	1.55	2.21
3.2	5	147	165	163	145	161	2.36	4.62	4.32	2.33	3.72
3.6	5	162	172	174	165	175	3.92	6.05	5.98	3.89	6.30
4.0	5	170	---	---	172	---	5.27	---	---	5.45	---

Table 4: The AT values (m/s) of participants determined via graded running on a treadmill

Subject	S-1	S-2	S-3	S-4	S-5
AT value	3.6	3.05	3.10	3.6	3.25

At the next steps prolonged constant-load exercise bouts were done by all participants.

The first and second constant-load exercise bouts consist of 30 minutes of running on treadmill at a velocity of 70% of AT and an elevation of 1% [LH: 70%AT, +1%] and 90% of AT and an 10% declination [HD: 90%AT, -10%], respectively.

Table 5: The velocity and duration of running of participants at the first and second bout of constant-load exercises

Subject	Step, Velocity & Duration	Low Intensity		High Intensity	
		Velocity (m/s)	Duration (min)	Velocity (m/s)	Duration (min)
S-1		2.50	30	3.24	30
S-2		2.14	30	2.75	30
S-3		2.17	30	2.79	30
S-4		2.50	30	3.24	30
S-5		2.28	30	2.95	30

Capillary blood sampling was done immediately before the start of the test at each bout of constant-load exercises and again one hour after the test. The serum were obtained and frozen until assayed. The serum count of H-FABP and Mb were measured by quantitative tests based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). Tables 6 and 7 showed the serum count of

those two above mentioned markers in low and high intensity exercises, respectively. Also the ratios of Mb over H-FABP have been showed in Table 8.

Table 6: The serum concentrations (ng/ml) of Mb and H-FABP in participants at the first step of test [LH: 70%AT, +1%]

Sampling's time & Marker Subject	Before the running		After running	
	Mb	H-FABP	Mb	H-FABP
S-1	28.02	6.25	39.26	5.87
S-2	33.18	7.74	49.28	7.91
S-3	25.00	6.25	31.02	9.63
S-4	32.13	5.93	39.63	6.78
S-5	87.33	10.70	47.54	12.25

Table 7: The serum concentrations (ng/ml) of Mb and H-FABP in participants at the second step of test [HD: 90%AT, -10%]

Sampling's time & Marker Subject	Before the running		After running	
	Mb	H-FABP	Mb	H-FABP
S-1	28.52	6.03	162.68	14.50
S-2	59.62	12.32	83.96	16.56
S-3	31.36	4.85	134.79	10.34
S-4	26.39	7.36	30.87	8.54
S-5	32.93	9.33	56.68	10.83

Table 8: The ratio of serum mean values of Mb over H-FABP in participants at both steps [LH: 70%AT, +1%; HD: 90%AT, -10%]

Intensity of exercise & Time of S* Subject	LH: 70%AT, +1%		HD: 90%AT, -10%	
	Before the running	After running	Before the running	After running
S-1	4.49	6.69	4.73	11.21
S-2	4.29	6.23	4.84	5.07
S-3	4.00	3.22	6.46	13.07
S-4	5.41	5.84	3.58	3.61
S-5	8.16	3.88	3.53	5.23

*Sampling

The serum mean values and the ratio of those two above mentioned markers have been indicated in Table 9 and Table 10, respectively.

In the present study we found no significant increase in serum count of either Mb or H-FABP in participants at the low intensity/ horizontal running of test. At the high intensity/ downhill running of test, we observed significant differences between the serum count of Mb and also H-FABP, pre and post running, in participants ($p < 0.05$) (Figure 9).

Table 9: The serum mean values of Mb and H-FABP in participants

Concentrations and time of sampling Marker - % AT	Mean \pm SD (ng/ml)	
	Before running	1 h after running
Mb [LH: 70% AT, +1%]	41.13 \pm 26.03	41.34 \pm 7.33
Mb [HD: 90%AT, -10%]	35.76 \pm 13.57	93.79 \pm 54.45 (*)
H-FABP [LH: 70% AT, +1%]	7.37 \pm 1.99	8.48 \pm 2.52
H-FABP [HD: 90%AT, -10%]	7.98 \pm 2.94	12.16 \pm 3.28 (*)

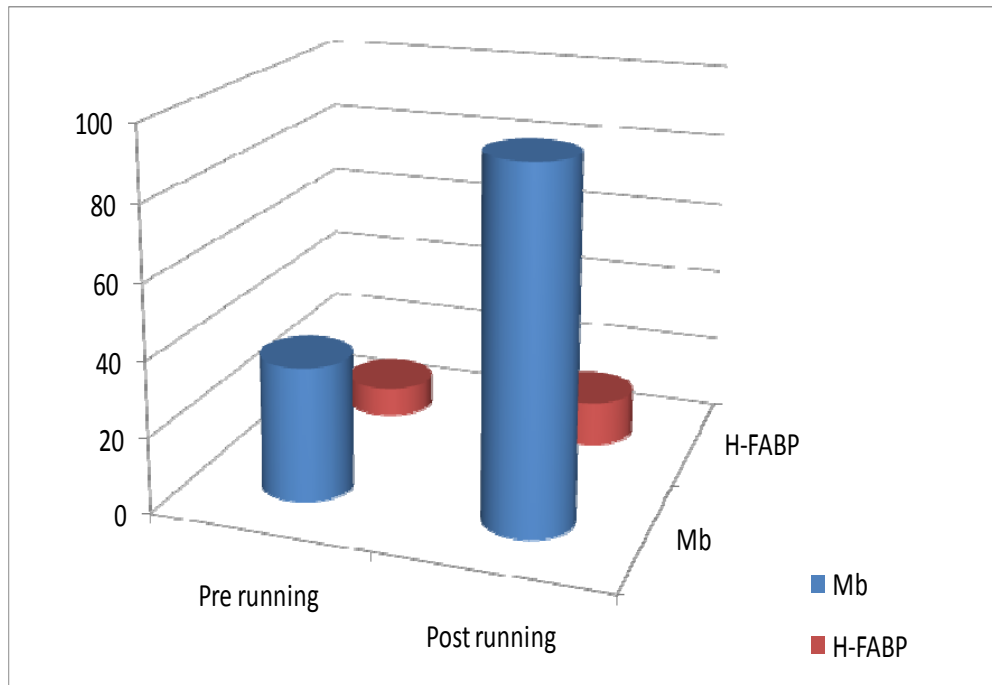


Figure 9: Comparison of serum mean values (ng/ml) of Mb and H-FABP in participants at the second step of test [HD: 90%AT, -10%]

There was also a significant difference between the ratio of serum count of Mb over H-FABP in participants at this step ($p < 0.05$) (Figure 10).

Table 10: The ratio of serum mean values of Mb over H-FABP in participants

Values at the relevant time	Ratio \pm SD	
	Before running	1 h after running
Ratio - % AT		
Mb/H-FABP [LH: 70% AT, +1%]	5.27 ± 1.69	5.17 ± 1.53
Mb/H-FABP [HD: 90%AT, -10%]	4.35 ± 0.73	$8.36 \pm 3.95 (*)$

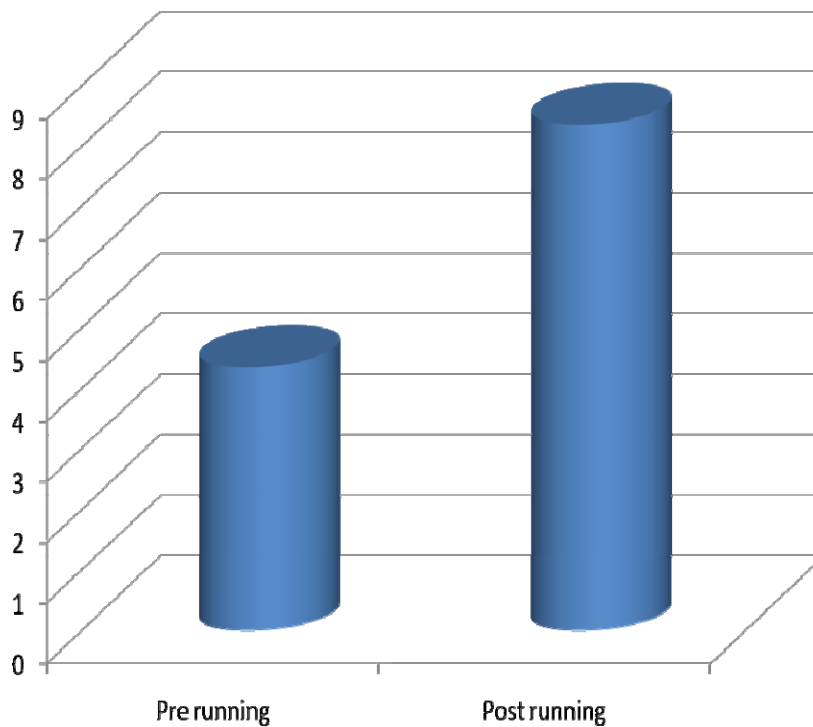


Figure 10: Comparison of the ratio of serum mean values of Mb over H-FABP in participants at the second step of test [HD: 90%AT, -10%]

5. Discussion

This thesis is divided into two main sections: theoretical part and practical part.

5.1. Theoretical part

In theoretical part some of muscle injury markers were reviewed and their characteristic issues summarized and advantages and disadvantages of those markers noted. In practical part of the current project, the effects of low and high intensity exercises on serum mean values of Mb and H-FABP were evaluated.

On the basis of this premise, in section 1 these two later markers were more widely reviewed than the other markers and especially some immunological assays for determining of them such as ELISA were reviewed.

A muscle has the capacity only to contract or not contract (relax). [4] When it contracts, the muscle always applies tension toward its middle. This may result in a concentric contraction, meaning the muscle shortens, or an eccentric contraction, meaning that while it applies tension the muscle gets longer because the resistance is greater than the tension. A muscle is said to contract isotonic (dynamically) when it either shortens or lengthens, and thus movement occurs, or isometrically (statically) when no movement occurs because the tension is not sufficient to overcome resistance against the muscle. Skeletal muscle is a remarkable tissue. Skeletal muscles are essential tissues that determine human performance. Without this important part of body, no one can dose any activity needed for daily life. Muscle injuries account for 30% of all sporting injuries. [109] Muscle injuries can be classified into those due to indirect trauma and those due to direct trauma. Indirect trauma injuries include delayed onset muscle soreness and muscle strain. Direct trauma injuries are subdivided into muscle contusion and muscle laceration. Muscle injuries may be assessed by several techniques. A musculoskeletal assessment requires a proper and thorough systematic

examination of the patient. A correct diagnosis depends on a knowledge of functional anatomy, an accurate patient history, diligent observation, and a thorough examination. The differential diagnosis process involves the use of clinical signs and symptoms, physical examination, a knowledge of pathology, and mechanisms of injury, provocative and palpation (motion) tests, and laboratory and diagnostic imaging techniques. It is only by a complete and systematic assessment that an accurate diagnosis can be made. [110]

The diagnosis of muscle injuries can be done through several techniques:

- Medical examination: Physical examination provides information about the injury and suggests appropriate further investigation.
- Imaging techniques: For example magnetic resonance imaging (MRI) provides major insights into the pathogenesis of disorders affecting the musculoskeletal system. MRI supplements investigations for the differential diagnosis of neuromuscular diseases. [111] Ultrasonography and MRI are both useful imaging modalities in defining the location, extent and severity of muscle injury. [110]
- Laboratory tests for blood analysis: These tests tell us about the severity or grade of lesion. [12, 112]

Advances in biological markers (biomarkers) have facilitated the assessment of associations between environmental exposures and potential health effects by improving accuracy and precision of measures and helping to identify high-risk groups in exposed populations. [20]

Obviously, the best treatment can be provided if the injury is diagnosed in its early stages. Early diagnosis of skeletal muscle injuries, one of the causes of athlete's inaction, and acute myocardial infarction (AMI), one of the most important mortality factors, are based on the recognition of muscle injury markers.

Muscle injury biochemical markers also play an important role in helping trainers make a good decision about the sportsmen and sportswomen.

The release of muscle content proteins is occurred after muscle injury. The both lysosomal (cathepsin C, D) and non-lysosomal proteases increase the rates of protein degradation and amino acid oxidation that occur during exercise. Protease calpain may initiate the exercise-induced changes in myofibrillar turnover and

organelle disassembly. In vitro, calpain cleaves a variety of substrates: receptor proteins, soluble and membrane associated enzymes, cytoskeletal proteins and myofibrillar proteins. Basically, the time and amount of extracellular release and clearance from plasma of biochemical markers of muscle damage depend on the biochemical characteristics of the molecule, the level of training, the type, intensity and duration of exercise, as well as on the period of recovery after training. [113] CK and Mb are routinely used to assess the injury in laboratory tests. Although some other markers such as fast myosin, sTnI, α -actin and H-FABP are also sometimes used in laboratory tests, their use in early assessment of muscle injuries is a challenge. In the current study we firstly reviewed some of muscle injury markers and their characteristic issues were summarized and then in practical part of project the serum concentrations of Mb and H-FABP were evaluated in athletes after low and high intensity exercises.

5.1.1. CK:

CK enzyme activity is detectable in muscle, heart and brain and also in serum. CK-MM, CK-BB and the hybrid form of CK-MB are characteristic for muscle, brain and heart, respectively. Monitoring of CK and characterisation of its isoenzymes is widely used in the diagnosis of cardiomyopathies, encephalopathies, and muscle disease. [114] CK is released slowly from skeletal muscle after exercise. [34] In veterinary medicine, serum CK activity is mostly used to assess skeletal muscle damage. Elevation of CK after exercise is a well-known phenomenon in healthy subjects, in humans as well as in animals. Eccentric activation of muscle has been widely implicated as the cause of large increases in plasma CK after exercise. [115] But CK is not an ideal marker for several reasons:

- Lack of tissue specificity: CK-MM is present in high concentrations in both skeletal and cardiac muscle. [116]
- Inability to reveal damage to specific skeletal fiber types.
- CK has low value in low concentration of glutathione.

CK activity may also be affected by different variables such as oestradiol levels, daily training, sex or type of sports discipline. [43] It is notable that the resting CK levels are higher in elite and professional athletes, but the significant increases of

CK occurring after exercise are usually lower in trained subjects than in untrained subjects. [113]

5.1.2. Mb:

Mb is a ubiquitous heme protein with a molecular weight of 17.800 kilodaltons, constituting about 2% of the total muscle protein. [116, 117] It has been suggested that Mb is an excellent marker of acute muscle damage or increased muscle membrane permeability. Though Mb has long been recognized as a cardiac marker, it has been limited in its use by poor specificity owing to its presence in skeletal muscle. A peak of Mb occurs shortly after damage and disappears rapidly. [39] However, Mb is also released in other disease states, including AMI, open heart surgery, muscular dystrophy, renal failure, shock and trauma. [118]

5.1.3. Fast myosin:

MHC is a structurally bound contractile protein of the thick filaments, and an increase in plasma MHC concentrations after exercise, therefore, indicates both membrane leakage and degradation of the contractile apparatus. [45] However, MHC shows a delayed increase after exercise-induced muscle injury. Therefore, MHC is not suitable for early diagnosis of muscle injuries.

5.1.4. α -actin:

α -actin is an abundant protein in the muscle, where it represents more than 20% of all cell proteins. [23] The presence of skeletal α -actin in blood stream may be a specific marker of skeletal muscle damage. Discrimination between skeletal and cardiac muscle damage can be done by the combined use of α -actin and Tnl.

5.1.5. sTnl:

sTnl is a regulatory protein that is only expressed in striated muscle fibers. [119] A marked increase of sTnl in patients with myoskeletal injury, with greater responses occurring for the bone than for the soft-tissue injuries. Marked increase in sTnl can be detected within 2-6 hours from the onset of exercise-induced muscle injury. [45] In contrast to all other available markers, sTnl is a protein

unique to skeletal muscle. sTnI may be an early marker for skeletal muscle damage. The release or clearance of sTnI can be affected by some conditions such as renal failure, compartment syndrome, and cardiac abnormalities.

5.1.6. H-FABP:

The soluble cytoplasm of skeletal and cardiac muscles and also some other tissue cells contains low-molecular mass proteins able to bind long chain unesterified fatty acids, that are called FABP. [120] FABP comprises 15% to 30% of cytoplasmic proteins. [125] In nine different identified cytoplasmic FABP types, H-FABP is most widely distributed within the organism. H-FABP appears to be a valid serum biomarker for the early diagnosis of: AMI, stroke, and acute muscle damage. It is a more suitable marker than CK-MB or Mb for early assessment of post-operative myocardial infarction. [118] The concentration of FABP in the plasma of healthy people is relatively low (2-6 µg/l). [120]

5.1.7. Mb/H-FABP ratio:

Mb and H-FABP, both are present in the heart and skeletal muscle. However, concentration of Mb is approximately two-fold lower in cardiac than skeletal muscle (2.5 and 4.0 mg/g wet weight of tissue) but the concentration of H-FABP in the heart is several-fold higher than that in the skeletal muscles (0.5 vs. 0.05-0.2 mg/g wet weight). [120, 121] In addition, the normal plasma concentration of H-FABP (<5 µg/l) is 10-15-fold lower than that of Mb (20-80 µg/l). Both proteins are released into plasma after injury at about the same time and in a ratio similar to the concentration of the proteins in the tissue of origin, therefore the measurement of the Mb/H-FABP ratio could be useful for discriminating between cardiac and skeletal muscle damage. However, the use of this ratio should not be a rigid criterion.

5.2. Comparison between above mentioned markers:

It is much interesting to make a diagram to compare the time course elevation of those above mentioned markers. To my knowledge there is no complete time course elevation relevant data for all those markers. Some accessible data will be shown below.

Sorichter and colleagues [45] compared the median peaks of CK, Mb, MHC and sTnI after downhill and level running to evaluate the muscle injury (Table 11).

Table 11: Comparison of peak values of muscle injury markers

Marker	Time to peak	Value	
		Downhill running	Level running
CK (activity) [U/l]	24 h	309	178
Mb [$\mu\text{g/l}$]	6 h	466	98
MHC [$\mu\text{U/l}$]	48 h	1021	501
sTnI [$\mu\text{g/l}$]	6 h	27.3	6.6

Eston, et al. [115] noted that the elevation of CK is started after 2 days of injury and its peak value, 1500-11000 U/l, reached after 4-5 days. Lippi and colleagues [113] observed that the peak value of Mb is reached after 3 hours and returned to normal range in 24 hours. Kirwan and colleagues [122] compared CK activity and Mb concentration after two unilateral isometric knee extension exercises. They observed different values for CK and Mb after 6 hours of those two above mentioned exercises. Martinez-Amat, et al. [23] compared the activity of CK and concentrations of Mb, cTnI and α -actin between muscle damage and control groups (Table 12).

Table 12: Comparison of muscle injury markers in muscle injury group and controls

Marker \ Group	Group	
	Muscle damage group	Control group
CK (IU/l)	2041.9 ± 2194.5 Range: 517 – 8943	105.5 ± 103.9 Range: 15 - 545
Mb (ng/ml)	891.815 ± 887.449 Range: 3.633 – 58.6	28.8697 ± 24 Range: 79.8 – 5.7
cTnl (ng/ml)	0.035 ± 0.031 Range: 0.001 – 0.160	0.0213 ± 0.0199 Range: 0.001 – 0.085
α-actin (ng/ml)	1968.51 ± 515.25 Range: 854 – 2.594	600.90 ± 532.97 Range: 0.00 – 1.359

Of course, Sorichter, et al. [64] evaluated muscle injury markers and showed their time course concentrations in a diagram. In their study peak FABP concentrations in individual subjects correlated with peak CK levels. Chu and colleagues [118] reported the time course elevation and peak of markers. As they reported the initial elevation for FABP, Mb, cTnl, CK-MB, occurred within 1.5-3, 1-3, 3-6 and 3-8 hours, and also the time to peak of those markers were 6-8, 5-8, 14-18 and 9-24 hours, respectively. Martinez-Amat, et al. [43] compared the muscle injury markers in sportspeople and non sportspeople (Table 13).

Table 13: Comparison of muscle injury markers between sportspeople and non sportspeople

Group Marker	Injured non-sportspeople	Injured sportspeople	Uninjured sportspeople	Uninjured non-sportspeople
Mb (ng/ml)	891.8±154.4	50.2±6.7	41.2±2.1	28.8±3.2
CK (IU/l)	2041.9±382.01	138.3±12.6	240.8±17.8	105.5±18.08
TnI (ng/ml)	0.035±0.005	0.016±0.001	0.017±0.001	0.021±0.003
α-actin (μg/ml)	37.4±1.3	10.4±1.8	3.9±0.7	0.2±0.03

Onuoha, et al. [119] compared the markers in three groups (Table 14).

Table 14: Comparison of three markers in three groups

Group Marker	Bone injury group	Soft-tissue injury group	Controls
sTnI (ng/ml)	16±2	10±2	2±0.9
CK (IU/l)	318±248	135±94	87±30
Mb (ng/l)	96±60	47±55	40±33

As mentioned above, there is not complete time course relevant data about the muscle injury markers. In existing data, there is a lot of diversity, due to some matters such as the type of muscle injured, intensity of exercise or physical activity and duration of exercise. Nevertheless, a diagram will be drawn below, showing the possibility and accuracy of use of those markers in early diagnosis of muscle injuries.

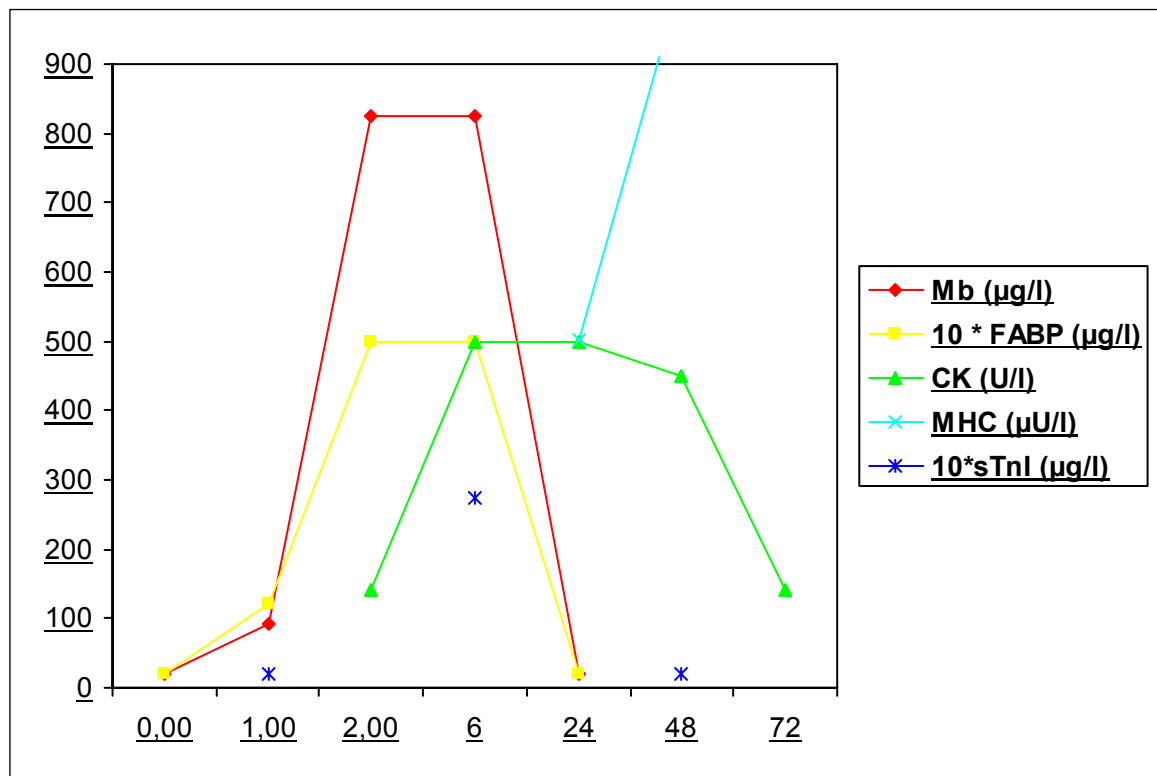


Figure 11: Comparison of the time course elevation of muscle injury markers

5.3. Practical part:

In practical part of the current study we observed that the serum concentrations of Mb and H-FABP and also the ratio of Mb over H-FABP is significantly different in subjects pre and 1 hour post running at high intensity/downhill running on treadmill [HD: 90%AT, -10%]. (Table15)

Table 15: Comparison of Mb and H-FABP after high intensity exercise in athletes

Time Marker	Pre running	1 h Post running
Mb	35.76 ± 13.57	93.79 ± 54.45
H-FABP	7.98 ± 2.94	12.16 ± 3.28
Mb/H-FABP	4.35 ± 0.73	8.36 ± 3.95

In practical part of the current study, the effects of low and high intensity exercises on plasma mean values of Mb and H-FABP were investigated.

The results of this part of the current study showed that the concentrations of both Mb and H-FABP increase on high intensity/ downhill running in serum of the subjects. All subjects were volunteer sports students and trainer who all passed the health examinations and considered well-trained athletes. Therefore, we assumed that the elevation of serum mean values of the two above mentioned markers arise from skeletal muscles.

To our knowledge this is the first time that the effects of low and high intensity exercises on serum concentrations of Mb and H-FABP, and subsequently assessment of muscle injury, have been evaluated in well trained athletes. It is well documented that eccentric muscle contractions produce skeletal muscle damage. [123] Sorichter and colleagues [64] showed for the first time that plasma FABP increases after physical exercise in healthy subjects and its pattern of release into and clearance from the blood is similar to that of Mb. Malek and colleagues [66] observed the increasing of H-FABP concentration on exercise in healthy subjects. As they reported the subjects had the H-FABP serum mean value of less than 7 ng/ml pre running, almost as much as we observed in our subjects pre running at both steps of our test.

Lippi and colleagues [113] measured some markers in their subjects. The mean values of Mb in the above mentioned showed to be elevated after a 21-km half-marathon (36 ± 3 , 102 ± 13 , 104 ± 14 ng/ml at pre, post and 3 h after running, respectively). It is almost as much as we measured in our subjects at the high intensity/ downhill running of test.

There is substantial evidence that eccentric contractions cause more damage than other types of contraction. An eccentric activation is the controlled lengthening of the muscle under tension. During downhill running the role of eccentric work of the anti-gravity muscles including knee extensors, muscles of the anterior and posterior tibial compartments and hip extensors, is accentuated. [115]

In the current study, as it was expected, after the high intensity/ downhill running on treadmill, the serum counts of evaluated markers were significantly higher than that before the running. The elevation of mean ratio of serum concentrations of Mb over H-FABP was in agreement with the results found by Sorichter and colleagues

[65]. However, the ratios calculated by them were higher than ours. This might be due to the fact that Sorichter excluded the samples in which both proteins (Mb and FABP) had not been increased to at least twice their baseline values when calculating the ratio as done by Nieuwenhoven. [56]

Moreover, the participants in Sorichter's study ran on a treadmill set at a 16% decline, thus likely producing more damage in muscles' cells than in our subjects as they ran on a treadmill set at a 10% decline.

A limitation of this study is the small size of subjects and therefore it seems that the findings in our current study should be confirmed on a larger group of voluntary subjects. In addition, we did not measure the serum value of any especial biochemical marker of heart muscle injury, such as cardiac troponin I (cTnI) to compare to the serum mean values of Mb and H-FABP.

In conclusion it can be suggested that simultaneous measurement of Mb and H-FABP and additionally express them as the ratio of Mb over H-FABP might give trainers the best information about the degree of damage of skeletal muscles in sportsmen or sportswomen.

Evidently, further investigation is needed to determine the real early assessment marker of muscle injury. An ideal marker of skeletal muscle injury would have the following characteristics [116] - It would:

- Be found in high concentration in skeletal muscle
- Not be found in other tissues, even in trace amounts or under pathological conditions
- Be released rapidly after skeletal muscle injury
- Be released in direct proportion to the extent of muscle injury
- Persist in plasma for several hours to provide a convenient diagnostic time window, but not so long that recurrent injury would not be identified
- Have a high sensitivity and specificity.

Although skeletal muscle injuries occurring in sports, exercises and physical activities restrict many of sport players and also other people who do sport recreationally to do their favourite sports and exercises, researchers have not concentrated on this field. Nieuwenhoven and colleagues [56] (1995) concluded that both Mb and H-FABP show a similar pattern of release into and clearance from plasma. In another study [67] (1994) they observed that the ratio of Mb

values over H-FABP is significantly different in cardiac muscle (4.5) compared to of that in skeletal muscles (21-73). They also reported that the ratio of the serum concentrations of Mb over H-FABP after myocardial injury is significantly different from that found when skeletal muscles are most likely injured, as the plasma ratios reflects the ratio in which the proteins occur in the injured tissue. It seems that these markers may open a new window to evaluate the skeletal muscle injuries. Yuan, et al. [65] (2003) compared the serum concentrations of CK to FABP in a junior group of rowers and concluded that FABP measurement will be more important for monitoring acute muscle damage among elite athletes after a single training session. Malek and colleagues [66] (2005) investigated whether H-FABP plasma concentration increases in healthy subjects after exercise. To my knowledge assessment of muscle injury markers for early evaluating of skeletal muscle injuries is challenging. Further studies are needed to investigate the whole potential role of blood markers in assessment of skeletal muscle injuries. Obviously achievement of optical blood skeletal muscle injury marker based on future researches in this field parallel to biochemical and immunological methods studies. Future studies will be aimed to access a very early assessment skeletal muscle injury marker in a very small amount of blood.

6. Summary

Muscle injuries are one of the most common traumas in sports and can be produced by intense or even moderate physical activity, especially eccentric exercise. It is important not to ignore even mild sport injuries, because this can exacerbate injuries and cause them to become far worse, much longer lasting and harder to treat. Common acute injuries to skeletal muscle can lead to significant pain and disability. Obviously pain and disability restrict the athletes to do their favourite sport and physical activities. Early affective diagnosis and treatment of these injuries is a challenge. The diagnosis of muscle injuries can be done through several techniques: medical examination, imaging techniques and laboratory tests for blood analysis. Obviously, the best treatment can be given if the injury is recognized in its early stages. The recognition of muscle injury markers is the most effective factor to achieve this goal.

In current study we reviewed some muscle injury markers published in recent decades, firstly.

Creatine kinase (CK) and myoglobin (Mb) are routinely used to evaluate the injuries in laboratory tests. High CK activity is detectable in muscle, heart and brain. CK-MM is the main isoenzyme in muscle.

It has been suggested that myoglobin (Mb) is an excellent marker of acute muscle damage or increased muscle membrane permeability.

Although fast myosin is a totally specific marker for skeletal muscle, it shows a delayed increase after exercise-induced muscle injury. It is therefore not suitable for early diagnosis of muscle injury.

α -actin is a new and reliable marker of skeletal muscle damage. Discrimination between skeletal and cardiac muscle damage can be done by the combined use of α -actin and troponin I (TnI).

Troponin I (TnI) is only expressed in striated muscle fibers. Slow and fast skeletal TnI (ssTnI and fsTnI) are produced in slow- and fast-twitch (ST and FT) fibers, respectively.

Fatty acid-binding protein (FABP) is one of the novel and promising plasma markers for detection of tissue injury. Heart-type (H)-FABP appears to be a valid serum biomarker for the early diagnosis of: AMI, stroke, and acute muscle damage.

The ratio of Mb over H-FABP is significantly different in cardiac muscle (4.5) compared to that in skeletal muscles (21-73). It seems that the ratio of serum mean values of Mb over H-FABP is a good marker to evaluate the cardiac and skeletal muscle injuries.

In practical part of the current study we evaluated the changes of serum mean values of Mb and H-FABP in a group of well trained athletes after low and high intensity exercises. The serum mean values of Mb and H-FABP and also the ratio of Mb over H-FABP were significantly increased after high intensity exercise in our subjects. It can be assumed that these two latter markers and expression of them as a ratio of Mb over H-FABP could give trainers a better diagnostic view of the players.

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Acknowledgment

First of all, I would like to dedicate this thesis to my father who was the first teacher of my life, when he taught me “to be patient in life”. Unfortunately he passed away, some years ago. God bless his soul.

I would like to take the opportunity to thank those people who spent their time and shared their knowledge for helping me to complete my thesis with the best possible result.

I am heartily thankful to my supervisor, Prof. Dr. med. Elke Zimmermann, whose encouragement and guidance from the initial to the final level enabled me to develop an understanding of the subject.

I am grateful to my next supervisor, Prof. Dr. Stephan Starischka, who kindly accepted to lead me as one of my supervisors.

I would like to extend my thanks to Dr. Reinhard von Piechowski who helped me to do the laboratory tests and provided comments and advices me.

I also would like to thank Dr. Christian Vobejda who always provided me useful advices.

Big thanks also go to all my colleagues, especially Marlies Trenner, Petra Borgsen and Tim Wortmann.

Finally, my special thanks to:

My mother for all your never-ending support and encouragement in my life. You showed me the best way of life.

Nahid, my wonderful wife, for all your support during these work-filled years in Germany, your encouragement and great helpful guidance.

Andisheh, our excellent and intelligent son. You are the sunshine of our life. We love you.

I am forever indebted to them.

Erklärung

Hiermit versichere ich, die vorliegende Dissertationsschrift mit dem Title

„Effects of low and high intensity exercises on serum mean values of myoglobin and heart-type fatty acid-binding protein in athletes“

Selbständig verfasst und keine, außer den angegebenen Hilfsmitteln und Quellen, verwendet zu haben. Zitate wurden als solche kenntlich gemacht. Die Dissertation lag weder in dieser noch in einer anderen Fassung einer anderen Universität oder Fakultät vor.

Bielefeld, 18.06.2010



Majid Mehdikhani